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(54) Title: **HUMAN BRAIN SODIUM-DEPENDENT INORGANIC PHOSPHATE COTRANSPORTER**

(57) Abstract

This invention describes a novel human brain Na⁺-dependent inorganic phosphate cotransporter, designated the hBNPI protein. This invention also encompasses nucleic acids encoding this protein, or a fragment thereof, as well as methods employing this protein and the nucleic acid compounds.

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HUMAN BRAIN SODIUM-DEPENDENT INORGANIC PHOSPHATE COTRANSPORTER

Inorganic phosphate (P_i), a charged anion, is
5 essential to bioenergetics, metabolic regulation, and bone
and membrane structure. It is well known that P_i homeostasis
in the body depends primarily on mechanisms that govern the
renal excretion of P_i into the glomerular filtrate and its
subsequent reabsorption against an electrochemical gradient
10 via brush-border epithelial cells located in the proximal
tubule of the kidney [J. Bonjour and J. Caverzasio, Reviews
in Physiological Pharmacology, 100:161-214 (1985); V.W.
Dennis, Phosphate homeostasis, in HANDBOOK OF PHYSIOLOGY, (S.
Shultz, ed. 1991) at pages 1785-1815.] This transepithelial
15 transport of P_i is mediated, in part, by a transport system
which is driven by the transmembrane Na^+ gradient across the
microvilli brush border membrane. However, it remains largely
unknown how cells transport and regulate necessary the
intracellular concentrations of P_i , and the molecular events
20 underlying this system. Experiments using isolated kidney
tubules or brush-border membranes have shown that P_i
transport is rather complex, regulated not only by
extracellular [P_i] but also by neurotransmitters such as
catecholamines (for review see V.W.Dennis, supra), and by a
25 variety of hormones and metabolic factors. Berndt and Knox,
"Renal Regulation of Phosphate Excretion", in, THE KIDNEY.
PHYSIOLOGY AND PATHOPHYSIOLOGY, (D.W. Seldin and G. Giebisch,
eds., 1991) at pages 1381-1396. Renal denervation, for
example, decreases sodium and phosphate reabsorption.
30 Norepinephrine released from nerve endings in proximity to
renal tubules acts on the proximal tubule to increase
phosphate reabsorption. In studies of isolated tubules,
however, dopamine is shown to inhibit phosphate and sodium
transport in the rabbit proximal tubule. Furthermore, several
35 studies also show that depletion of extracellular P_i or
increased circulating levels of parathyroid hormone alter the
activity and expression of transporter molecules or both.

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Several recent reports have demonstrated that Pi homeostasis significantly affects the central nervous system (CNS). Phosphate/calcium alterations in serum, for example, have been implicated in the etiology and pathogenesis of 5 Alzheimer's diseases. Depletion of high energy phosphates (phosphocreatine) and ATP is thought to be part of the final common pathway mediating excitotoxic neuronal cell death secondary to a wide variety of insults. Tight coupling between Pi transport and ATP production has been observed in 10 many cells and tissues. Chronic Pi depletion in vivo is associated with a significant reduction in the ATP content of polymorphonuclear leukocytes, platelets, and various tissues including kidney, heart, and skeletal muscle. A similar observation has been made in cultured peripheral vagal 15 nerves. This reduction in intracellular ATP has been shown to be a direct consequence of the decrease in intracellular Pi which occurs following Pi depletion. In addition to its possible role in ATP biosynthesis, several lines of evidence have suggested that Pi may be involved in neuronal signalling 20 events. In this regard, a study using brain tissue has recently shown that physiological concentrations of Pi can enhance the ATP-dependent binding of Ca⁺⁺ to brain microsomes, resulting in a larger intracellular pool of Ca⁺⁺ releasable by inositol triphosphate. Our recent work have 25 demonstrated that >90% Pi transport in cortical neurons, which displays similar kinetic parameters to those reported for cultured kidney proximal tubule epithelial cells and membrane vesicles, are sodium dependent and that this Na⁺-dependent transport system is regulated through a Na⁺- 30 dependent Pi cotransporter. B. Ni, et al., Proceedings of the National Academy of Sciences (USA), 91:5607-5611 (1994).

The present invention describes the cloning and characterization of a human brain Na⁺-dependent Pi cotransporter which is selectively expressed in discrete 35 populations of neurons and glia. Fluorescent *in situ* hybridization (FISH) analysis demonstrates that this Na⁺-dependent Pi cotransporter is located in chromosome 19

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(19q13.3) which has been linked to susceptible gene(s) for late onset Alzheimer's disease. M. Mullan and F. Crawford, Trends in Neurological Sciences, 16, 398-403 (1993). The characterization and treatment of physiological disorders is 5 hereby furthered.

This invention provides an isolated amino acid compound useful as a human brain sodium-dependent inorganic phosphate cotransporter, said compound comprising the amino 10 acid sequence

	Met	Glu	Phe	Arg	Gln	Glu	Glu	Phe	Arg	Lys	Leu	Ala	Gly	Arg	Ala	Leu
	1					5				10					15	
15	Gly	Lys	Leu	His	Arg	Leu	Leu	Glu	Lys	Arg	Gln	Glu	Gly	Ala	Glu	Thr
						20			25						30	
20	Leu	Glu	Leu	Ser	Ala	Asp	Gly	Arg	Pro	Val	Thr	Thr	Gln	Thr	Arg	Asp
						35			40				45			
25	Pro	Pro	Val	Val	Asp	Cys	Thr	Cys	Phe	Gly	Leu	Pro	Arg	Arg	Tyr	Ile
						50			55			60				
30	Ile	Ala	Ile	Met	Ser	Gly	Leu	Gly	Phe	Cys	Ile	Ser	Phe	Gly	Ile	Arg
						65			70			75			80	
35	Cys	Asn	Leu	Gly	Val	Ala	Ile	Val	Ser	Met	Val	Asn	Asn	Ser	Thr	Thr
						85			90			95				
40	His	Arg	Gly	Gly	His	Val	Val	Val	Gln	Lys	Ala	Gln	Phe	Ser	Trp	Asp
						100			105			110				
45	Pro	Glu	Thr	Val	Gly	Leu	Ile	His	Gly	Ser	Phe	Phe	Trp	Gly	Tyr	Ile
						115			120			125				
50	Val	Thr	Gln	Ile	Pro	Gly	Gly	Phe	Ile	Cys	Gln	Lys	Phe	Ala	Ala	Asn
						130			135			140				
55	Arg	Val	Phe	Gly	Phe	Ala	Ile	Val	Ala	Thr	Ser	Thr	Leu	Asn	Met	Leu
						145			150			155			160	
60	Ile	Pro	Ser	Ala	Ala	Arg	Val	His	Tyr	Gly	Cys	Val	Ile	Phe	Val	Arg
						165			170			175				
65	Ile	Leu	Gln	Gly	Leu	Val	Glu	Gly	Val	Thr	Tyr	Pro	Ala	Cys	His	Gly
						180			185			190				
70	Ile	Trp	Ser	Lys	Trp	Ala	Pro	Pro	Leu	Glu	Arg	Ser	Arg	Leu	Ala	Thr
						195			200			205				

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Thr Ala Phe Cys Gly Ser Tyr Ala Gly Ala Val Val Ala Met Pro Leu
210 215 220

5 Ala Gly Val Leu Val Gln Tyr Ser Gly Trp Ser Ser Val Phe Tyr Val
225 230 235 240

Tyr Gly Ser Phe Gly Ile Phe Trp Tyr Leu Phe Trp Leu Leu Val Ser
245 250 255

10 Tyr Glu Ser Pro Ala Leu His Pro Ser Ile Ser Glu Glu Glu Arg Lys
260 265 270

Tyr Ile Glu Asp Ala Ile Gly Glu Ser Ala Lys Leu Met Asn Pro Leu
275 280 285

15 Thr Lys Phe Ser Thr Pro Trp Arg Arg Phe Phe Thr Ser Met Pro Val
290 295 300

20 Tyr Ala Ile Ile Val Ala Asn Phe Cys Arg Ser Trp Thr Phe Tyr Leu
305 310 315 320

Leu Leu Ile Ser Gln Pro Asp Tyr Phe Glu Glu Val Phe Gly Phe Glu
325 330 335

25 Ile Ser Lys Val Gly Leu Val Ser Ala Leu Pro His Leu Val Met Thr
340 345 350

Ile Ile Val Pro Ile Gly Gly Gln Ile Ala Asp Phe Leu Arg Ser Arg
355 360 365

30 Arg Ile Met Ser Thr Thr Asn Val Arg Lys Leu Met Asn Cys Gly Gly
370 375 380

35 Phe Gly Met Glu Ala Thr Leu Leu Leu Val Val Gly Tyr Ser His Ser
385 390 395 400

Lys Gly Val Ala Ile Ser Phe Leu Val Leu Ala Val Gly Phe Ser Gly
405 410 415

40 Phe Ala Ile Ser Gly Phe Asn Val Asn His Leu Asp Ile Ala Pro Arg
420 425 430

Tyr Ala Ser Ile Leu Met Gly Ile Ser Asn Gly Val Gly Thr Leu Ser
435 440 445

45 Gly Met Val Cys Pro Ile Ile Val Gly Ala Met Thr Lys His Lys Thr
450 455 460

50 Arg Glu Glu Trp Gln Tyr Val Phe Leu Ile Ala Ser Leu Val His Tyr
465 470 475 480

Gly Gly Val Ile Phe Tyr Gly Val Phe Ala Ser Gly Glu Lys Gln Pro
485 490 495

55 Trp Ala Glu Pro Glu Glu Met Ser Glu Glu Lys Cys Gly Phe Val Gly

- 5 -

	500	505	510
	His Asp Gln Leu Ala Gly Ser Asp Asp Ser Glu Met Glu Asp Glu Ala		
	515	520	525
5	Glu Pro Pro Gly Ala Pro Pro Ala Pro Pro Pro Ser Tyr Gly Ala Thr		
	530	535	540
10	His Ser Thr Phe Gln Pro Pro Arg Pro Pro Pro Val Arg Asp Tyr		
	545	550	555
			560

hereinafter designated as SEQ ID NO:2.

The invention also provides an isolated nucleic acid compound that comprises a nucleic acid sequence which encodes for the amino acid compounds provided. Particularly this invention provides the isolated nucleic acid compound having the sequence

20	CGATAAGCTT GATATCGAAT TCCGGACTCT TGCTCGGGCG CCTTAACCCG GCGTTCGGTT	60
	CATCCCGCAG CGCCAGTTCT GCTTACCAAA AGTGGCCCAC TAGGCACTCG CATTCCACGC	120
	CCGGCTCCAC GCCAGCGAGC CGGGCTTCTT ACCCATTAA AGTTTGAGAA TAGTTGAGA	180
25	TCGTTTCGGC CCCAAGACCT CTAATCATTC GCTTTACCGG ATAAAACCTGC GTGGCGGGGG	240
	TGCGTCGGGT CTGCGAGAGC GCCAGCTATC CTGAGGGAAA CTTCGGAGGG AACCAAGCTAC	300
	TAGATGGTTC GATTAGTCTT TCGCCCCAT ACCCAGGTCTG GACGACCGAT TTGCACGTCA	360
30	GGACCGCTAC GGACCTCCAC CAGAGTTTCC TCTGGCTTCG CCCTGCCAG GCGATCGGCG	420
	GGGGGGACCC GCAGGGTGAC CGGCAGCAGG AGCCGCCACC ATG GAG TTC CGC CAG Met Glu Phe Arg Gln	475
35	1 5	
	GAG GAG TTT CCG AAG CTA GCG GGT CGT GCT CTC GGG AAG CTG CAC CGC Glu Glu Phe Arg Lys Leu Ala Gly Arg Ala Leu Gly Lys Leu His Arg	523
40	10 15 20	
	CTT CTG GAG AAG CGG CAG GAA GGC GCG GAG ACG CTG GAG CTG AGT GCG Leu Leu Glu Lys Arg Gln Glu Gly Ala Glu Thr Leu Glu Leu Ser Ala	571
	25 30 35	
45	GAT GGG CGC CCG GTG ACC ACG CAG ACC CGG GAC CCG CCG GTG GTG GAC Asp Gly Arg Pro Val Thr Thr Gln Thr Arg Asp Pro Pro Val Val Asp	619
	40 45 50	
50	TGC ACC TGC TTC GGC CTC CCT CGC CGC TAC ATT ATC GCC ATC ATG AGT Cys Thr Cys Phe Gly Leu Pro Arg Arg Tyr Ile Ile Ala Ile Met Ser	667
	55 60 65	

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	GGT CTG GGC TTC TGC ATC AGC TTT GGC ATC CGC TGC AAC CTG GGC GTG Gly Leu Gly Phe Cys Ile Ser Phe Gly Ile Arg Cys Asn Leu Gly Val 70 75 80 85	715
5	GCC ATC GTC TCC ATG GTC AAT AAC AGC ACG ACC CAC CGC GGG GGC CAC Ala Ile Val Ser Met Val Asn Asn Ser Thr Thr His Arg Gly Gly His 90 95 100	763
10	GTG GTG GTG CAG AAA GCC CAG TTC AGC TGG GAT CCA GAG ACT GTC GGC Val Val Val Gln Lys Ala Gln Phe Ser Trp Asp Pro Glu Thr Val Gly 105 110 115	811
15	CTC ATA CAC GGC TCC TTT TTC TGG GGC TAC ATT GTC ACT CAG ATT CCA Leu Ile His Gly Ser Phe Phe Trp Gly Tyr Ile Val Thr Gln Ile Pro 120 125 130	859
20	GGA GGA TTT ATC TGT CAA AAA TTT GCA GCC AAC AGA GTT TTC GGC TTT Gly Gly Phe Ile Cys Gln Lys Phe Ala Ala Asn Arg Val Phe Gly Phe 135 140 145	907
25	GCT ATT GTG GCA ACA TCC ACT CTA AAC ATG CTG ATC CCC TCA GCT GCC Ala Ile Val Ala Thr Ser Thr Leu Asn Met Leu Ile Pro Ser Ala Ala 150 155 160 165	955
30	CGC GTC CAC TAT GGC TGT GTC ATC TTC GTG AGG ATC CTG CAG GGG TTG Arg Val His Tyr Gly Cys Val Ile Phe Val Arg Ile Leu Gln Gly Leu 170 175 180	1003
35	GTA GAG GGG GTC ACA TAC CCC GCC TGC CAT GGG ATC TGG AGC AAA TGG Val Glu Gly Val Thr Tyr Pro Ala Cys His Gly Ile Trp Ser Lys Trp 185 190 195	1051
40	GCC CCA CCC TTA GAA CGG AGT CGC CTG GCG ACG ACA GCC TTT TGT GGT Ala Pro Pro Leu Glu Arg Ser Arg Leu Ala Thr Thr Ala Phe Cys Gly 200 205 210	1099
45	TCC TAT GCT GGG GCG GTG GTC GCG ATG CCC CTC GCC GGG GTC CTT GTG Ser Tyr Ala Gly Ala Val Ala Met Pro Leu Ala Gly Val Leu Val 215 220 225	1147
50	CAG TAC TCA GGA TGG AGC TCT GTT TTC TAC GTC TAC GGC AGC TTC GGG Gln Tyr Ser Gly Trp Ser Ser Val Phe Tyr Val Tyr Gly Ser Phe Gly 230 235 240 245	1195
55	ATC TTC TGG TAC CTG TTC TGG CTG CTC GTC TCC TAC GAG TCC CCC GCG Ile Phe Trp Tyr Leu Phe Trp Leu Leu Val Ser Tyr Glu Ser Pro Ala 250 255 260	1243
	CTG CAC CCC AGC ATC TCG GAG GAG CGC AAG TAC ATC GAG GAC GCC Leu His Pro Ser Ile Ser Glu Glu Glu Arg Lys Tyr Ile Glu Asp Ala 265 270 275	1291
	ATC GGA GAG AGC GCG AAA CTC ATG AAC CCC CTC ACG AAG TTT AGC ACT Ile Gly Glu Ser Ala Lys Leu Met Asn Pro Leu Thr Lys Phe Ser Thr	1339

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	280	285	290		
5	CCC TGG CGG CGC TTC TTC ACG TCT ATG CCA GTC TAT GCC ATC ATC GTG Pro Trp Arg Arg Phe Phe Thr Ser Met Pro Val Tyr Ala Ile Ile Val 295	300	305	1387	
10	GCC AAC TTC TGC CGC AGC TGG ACG TTC TAC CTG CTG CTC ATC TCC CAG Ala Asn Phe Cys Arg Ser Trp Thr Phe Tyr Leu Leu Leu Ile Ser Gln 310	315	320	325	1435
15	CCC GAC TAC TTC GAA GAA GTG TTC GGC TTC GAG ATC AGC AAG GTA GGC Pro Asp Tyr Phe Glu Glu Val Phe Gly Phe Glu Ile Ser Lys Val Gly 330	335	340	1483	
20	CTG GTG TCC GCG CTG CCC CAC CTG GTC ATG ACC ATC ATC GTG CCC ATC Leu Val Ser Ala Leu Pro His Leu Val Met Thr Ile Ile Val Pro Ile 345	350	355	1531	
25	GGC GGC CAG ATC GCG GAC TTC CTG CGG AGC CGC CGC ATC ATG TCC ACC Gly Gly Gln Ile Ala Asp Phe Leu Arg Ser Arg Arg Ile Met Ser Thr 360	365	370	1579	
30	ACC AAC GTG CGC AAG TTG ATG AAC TGC GGA GGC TTC GGC ATG GAA GCC Thr Asn Val Arg Lys Leu Met Asn Cys Gly Gly Phe Gly Met Glu Ala 375	380	385	1627	
35	ACG CTG CTG TTG GTG GTC GGC TAC TCG CAC TCC AAG GGC GTG GCC ATC Thr Leu Leu Leu Val Val Gly Tyr Ser His Ser Lys Gly Val Ala Ile 390	395	400	405	1675
40	TCC TTC CTG GTC CTA GCC GTG GGC TTC AGC GGC TTC GCC ATC TCT GGG Ser Phe Leu Val Leu Ala Val Gly Phe Ser Gly Phe Ala Ile Ser Gly 410	415	420	1723	
45	TTC AAC GTG AAC CAC CTG GAC ATA GCC CCG CGC TAC GCC AGC ATC CTC Phe Asn Val Asn His Leu Asp Ile Ala Pro Arg Tyr Ala Ser Ile Leu 425	430	435	1771	
50	ATG GGC ATC TCC AAC GGC GTG GGC ACA CTG TCG GGC ATG GTG TGC CCC Met Gly Ile Ser Asn Gly Val Gly Thr Leu Ser Gly Met Val Cys Pro 440	445	450	1819	
55	ATC ATC GTG GGG GCC ATG ACT AAG CAC AAG ACT CGG GAG GAG TGG CAG Ile Ile Val Gly Ala Met Thr Lys His Lys Thr Arg Glu Glu Trp Gln 455	460	465	1867	
50	TAC GTG TTC CTA ATT GCC TCC CTG GTG CAC TAT GGA GGT GTC ATC TTC Tyr Val Phe Leu Ile Ala Ser Leu Val His Tyr Gly Gly Val Ile Phe 470	475	480	485	1915
55	TAC GGG GTC TTT GCT TCT GGA GAG AAG CAG CCG TGG GCA GAG CCT GAG Tyr Gly Val Phe Ala Ser Gly Glu Lys Gln Pro Trp Ala Glu Pro Glu 490	495	500	1963	
55	GAG ATG AGC GAG GAG AAG TGT GGC TTC GTT GCC CAT GAC CAG CTG GCT			2011	

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	Glu Met Ser Glu Glu Lys Cys Gly Phe Val Gly His Asp Gln Leu Ala	
	505	510
	515	
5	GGC AGT GAC GAC AGC GAA ATG GAG GAT GAG GCT GAG CCC CCG GGG GCA Gly Ser Asp Asp Ser Glu Met Glu Asp Glu Ala Glu Pro Pro Gly Ala	2059
	520	525
	530	
10	CCC CCT GCA CCC CCG CCC TCC TAT GGG GCC ACA CAC AGC ACA TTT CAG Pro Pro Ala Pro Pro Pro Ser Tyr Gly Ala Thr His Ser Thr Phe Gln	2107
	535	540
	545	
15	CCC CCC AGG CCC CCA CCC CCT GTC CGG GAC TAC TGA CCATGTGCCT Pro Pro Arg Pro Pro Pro Val Arg Asp Tyr *	2153
	550	555
	560	
	CCCACTGAAT GGCAAGTTCC AGGACCTCCA TTCCACTCAT CTCTGGCCTG AGTGACAGTG	2213
	TCAAGGAACC CTGCTCCTCT CTGTCCTGCC TCAGGCCTAA GAAGCACTCT CCCTTGTTCC	2273
20	CAGTGCTGTC AAATCCTCTT TCCTTCCCAA TTGCCTCTCA GGGGTAGTGA AGCTGCAGAC	2333
	TGACAGTTTC AAGGATAACCC AAATTCCCTT AAAGGTTCCCC TCTCCACCCG TTCTGCCTCA	2393
25	GTGGTTCAA ATCTCTCCTT TCAGGGCTTT ATTTGAATGG ACAGTTCGAC CTCTTACTCT	2453
	CTCTTGTGGT TTTGAGGCAC CCACACCCCC CGCTTCCTT TATCTCCAGG GACTCTCAGG	2513
	CTAACCTTG AGATCACTCA GCTCCCACCT CCTTCAGAA AAATTCAAGG TCCTCCTCTA	2573
30	GAAGTTCAA ATCTCTCCCA ACTCTGTTCT GCATCTCCA GATTGGTTTA ACCAATTACT	2633
	CGTCCCCGCC ATTCCAGGGA TTGATTCTCA CCAGCGTTTC TGATGGAAAA TGGCGGGAAT	2693
	TCCTGCAGCC CGGGGGATCC ACT	2716
35	which is hereinafter designated as SEQ ID NO:1.	

This invention also provides recombinant nucleic acid vectors comprising nucleic acids encoding SEQ ID NO:2. This invention also encompasses recombinant DNA vectors which comprise the isolated DNA sequence which is SEQ ID NO:1.

The present invention also provides assays for determining the efficacy and adverse reaction profile of agents useful in the treatment or prevention of disorders associated with an inappropriate stimulation of a human brain Na⁺-dependent inorganic phosphate cotransporter.

The terms and abbreviations used in this document have their normal meanings unless otherwise designated. For

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example "°C" refers to degrees Celsius; "N" refers to normal or normality; "mmol" refers to millimole or millimoles; "g" refers to gram or grams; "ml" means milliliter or milliliters; "M" refers to molar or molarity; "μg" refers to 5 microgram or micrograms; and "μl" refers to microliter or microliters.

All nucleic acid sequences, unless otherwise designated, are written in the direction from the 5' end to the 3' end, frequently referred to as "5' to 3'".

10 All amino acid or protein sequences, unless otherwise designated, are written commencing with the amino terminus ("N-terminus") and concluding with the carboxy terminus ("C-terminus").

"Base pair" or "bp" as used herein refers to DNA or 15 RNA. The abbreviations A,C,G, and T correspond to the 5'-monophosphate forms of the deoxyribonucleosides (deoxy)adenine, (deoxy)cytidine, (deoxy)guanine, and (deoxy)thymine, respectively, when they occur in DNA molecules. The abbreviations U,C,G, and T correspond to the 20 5'-monophosphate forms of the ribonucleosides uracil, cytidine, guanine, and thymine, respectively when they occur in RNA molecules. In double stranded DNA, base pair may refer to a partnership of A with T or C with G. In a DNA/RNA, heteroduplex base pair may refer to a partnership of 25 A with U or C with G. (See the definition of "complementary", infra.)

The terms "digestion" or "restriction" of DNA refers to the catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the 30 DNA ("sequence-specific endonucleases"). The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements were used as would be known to one of ordinary skill in the art. Appropriate buffers and substrate amounts 35 for particular restriction enzymes are specified by the manufacturer or can be readily found in the literature.

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"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments. Unless otherwise provided, ligation may be accomplished using known buffers and conditions with a DNA 5 ligase, such as T4 DNA ligase.

The term "plasmid" refers to an extrachromosomal (usually) self-replicating genetic element. Plasmids are generally designated by a lower case "p" preceded and/or followed by letters and/or numbers. The starting plasmids 10 herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accordance with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled 15 artisan.

The term "reading frame" means the nucleotide sequence from which translation occurs "read" in triplets by the translational apparatus of transfer RNA (tRNA) and ribosomes and associated factors, each triplet corresponding 20 to a particular amino acid. A base pair insertion or deletion (termed a frameshift mutation) may result in two different proteins being coded for by the same DNA segment. To insure against this, the triplet codons corresponding to the desired polypeptide must be aligned in multiples of three 25 from the initiation codon, i.e. the correct "reading frame" being maintained.

"Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA 30 molecule to which one or more additional DNA segments can or have been added.

The term "recombinant DNA expression vector" as used herein refers to any recombinant DNA cloning vector in which a promoter to control transcription of the inserted DNA 35 has been incorporated.

The term "expression vector system" as used herein refers to a recombinant DNA expression vector in combination

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with one or more trans-acting factors that specifically influence transcription, stability, or replication of the recombinant DNA expression vector. The trans-acting factor may be expressed from a co-transfected plasmid, virus, or
5 other extrachromosomal element, or may be expressed from a gene integrated within the chromosome.

"Transcription" as used herein refers to the process whereby information contained in a nucleotide sequence of DNA is transferred to a complementary RNA
10 sequence.

The term "transfection" as used herein refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled
15 artisan, for example, calcium phosphate co-precipitation, and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

The term "transformation" as used herein means the
20 introduction of DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Methods of transforming bacterial and eukaryotic hosts are well known in the art, many of which methods, such as nuclear injection, protoplast fusion or by
25 calcium treatment using calcium chloride are summarized in J. Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, (1989).

The term "translation" as used herein refers to the process whereby the genetic information of messenger RNA is used to specify and direct the synthesis of a polypeptide
30 chain.

The term "vector" as used herein refers to a nucleic acid compound used for the transformation of cells in gene manipulation bearing polynucleotide sequences corresponding to appropriate protein molecules which when
35 combined with appropriate control sequences confer specific properties on the host cell to be transformed. Plasmids, viruses, and bacteriophage are suitable vectors. Artificial

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vectors are constructed by cutting and joining DNA molecules from different sources using restriction enzymes and ligases. The term "vector" as used herein includes Recombinant DNA cloning vectors and Recombinant DNA expression vectors.

5 The terms "complementary" or "complementarity" as used herein refers to pair of bases, purines and pyrimidines, that associate through hydrogen bonding in double stranded nucleic acid. The following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and 10 uracil.

The term "hybridization" as used herein refers to a process in which a strand of nucleic acid joins with a complementary strand through base pairing. The conditions employed in the hybridization of two non-identical, but very 15 similar, complementary nucleic acids varies with the degree of complementarity of the two strands and the length of the strands. Such techniques and conditions are well known to practitioners in this field.

20 "Isolated amino acid sequence" refers to any amino acid sequence, however constructed or synthesized, which is locationally distinct from the naturally occurring sequence.

"Isolated DNA compound" refers to any DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location in genomic DNA.

25 "Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or 30 synthetic elongation.

The term "promoter" refers to a DNA sequence which directs transcription of DNA to RNA.

A "probe" as used herein is a nucleic acid compound or a fragment thereof which hybridizes with a nucleic acid 35 compound which encodes either the entire sequence SEQ ID NO:2, a sequence complementary to SEQ ID NO:2, or a part thereof.

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The term "stringency" refers to a set of hybridization conditions which may be varied in order to vary the degree of nucleic acid affinity for other nucleic acid. (See the definition of "hybridization", supra.)

5 The term "antigenically distinct" as used herein refers to a situation in which antibodies raised against an epitope of the proteins of the present invention, or a fragment thereof, may be used to differentiate between the proteins of the present invention and other brain Na⁺-dependent inorganic phosphate cotransporter subtypes. This term may also be employed in the sense that such antibodies may be used to differentiate between the human hBNPI protein protein and analogous proteins derived from other species.

10 The term "PCR" as used herein refers to the widely-known polymerase chain reaction employing a thermally-stable polymerase.

15 Skilled artisans will recognize that the proteins of the present invention can be synthesized by a number of different methods. All of the amino acid compounds of the invention can be made by chemical methods well known in the art, including solid phase peptide synthesis, or recombinant methods. Both methods are described in U.S. Patent 4,617,149, the entirety of which is herein incorporated by reference.

20 The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. See, e.g., H. Dugas and C. Penney, BIOORGANIC CHEMISTRY, (1981) at pages 54-92. For examples, 25 peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City California) and synthesis cycles supplied by Applied Biosystems. Protected amino acids, such as t-butoxycarbonyl-protected amino acids, and other reagents are commercially available from many chemical supply houses.

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Sequential *t*-butoxycarbonyl chemistry using double couple protocols are applied to the starting *p*-methyl benzhydryl amine resins for the production of C-terminal carboxamides. For the production of C-terminal acids, the 5 corresponding pyridine-2-aldoxime methiodide resin is used. Asparagine, glutamine, and arginine are coupled using preformed hydroxy benzotriazole esters. The following side chain protection may be used:

Arg, Tosyl
10 Asp, cyclohexyl
Glu, cyclohexyl
Ser, Benzyl
Thr, Benzyl
Tyr, 4-bromo carbobenzoxy
15 Removal of the *t*-butoxycarbonyl moiety (deprotection) may be accomplished with trifluoroacetic acid (TFA) in methylene chloride. Following completion of the synthesis the peptides may be deprotected and cleaved from the resin with anhydrous hydrogen fluoride containing 10%
20 meta-cresol. Cleavage of the side chain protecting group(s) and of the peptide from the resin is carried out at zero degrees centigrade or below, preferably -20°C for thirty minutes followed by thirty minutes at 0°C.

After removal of the hydrogen fluoride, the 25 peptide/resin is washed with ether, and the peptide extracted with glacial acetic acid and then lyophilized. Purification is accomplished by size-exclusion chromatography on a Sephadex G-10 (Pharmacia) column in 10% acetic acid.

The proteins of the present invention may also be 30 produced by recombinant methods. Recombinant methods are preferred if a high yield is desired. A general method for the construction of any desired DNA sequence is provided in J. Brown, et al., *Methods in Enzymology*, 68:109 (1979). See also, J. Sambrook, et al., supra.

35 The basic steps in the recombinant production of desired proteins are:

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- a) construction of a synthetic or semi-synthetic DNA encoding the protein of interest;
- 5 b) integrating said DNA into an expression vector in a manner suitable for the expression of the protein of interest, either alone or as a fusion protein;
- 10 c) transforming an appropriate eukaryotic or prokaryotic host cell with said expression vector,
- 15 d) culturing said transformed or transfected host cell in a manner to express the protein of interest; and
- 20 e) recovering and purifying the recombinantly produced protein of interest.

In general, prokaryotes are used for cloning of DNA sequences in constructing the vectors of this invention. Prokaryotes may also be employed in the production of the protein of interest. For example, the Escherichia coli K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of E. coli which may be used (and their relevant genotypes) include the following.

30	<u>Strain</u>	<u>Genotype</u>
	DH5 α	F $^-$ (φ 80dlacZ Δ M15), Δ (lacZYA-argF)U169 supE44, λ $^-$, hsdR17(r K $^+$, m K $^+$), recA1, endA1, gyrA96, thi-1, relA1

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HB101 supE44, hsdS20(r_B⁻ m_B⁻), recA13, ara-14, proA2 lacY1, galK2, rpsL20, xyl-5, mtl-1, mcrB, mrr

5 JM109 recA1, e14⁻(mcrA), supE44, endA1, hsdR17(r_K⁻, m_K⁺), gyrA96, relA1, thi-1, Δ(lac-proAB), F'[traD36, proAB+ lacI^q, lacZΔM15]

10 RR1 supE44, hsdS20(r_B⁻ m_B⁻), ara-14 proA2, lacY1, galK2, rpsL20, xyl-5, mtl-5

χ1776 F⁻, ton, A53, dapD8, minA1, supE42 (glnV42), Δ(gal-uvrB)40, minB2, rfb-2, gyrA25, thyA142, oms-2, metC65, oms-1, Δ(bioH-asd)29, cycB2, cycA1, hsdR2, λ⁻

20 294 endA, thi⁻, hsr⁻, hsm_K⁺ (U.S. Patent 4,366,246)

LE392 F⁻, hsdR514 (r⁻m⁻), supE44, supF58, lacY1, or Δlac(I-Y)6, galK2, glaT22, metB1, trpR55, λ⁻

25 These strains are all commercially available from suppliers such as: Bethesda Research Laboratories, Gaithersburg, Maryland 20877 and Stratagene Cloning Systems, La Jolla, California 92037; or are readily available to the public from sources such as the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 10852-1776.

30 Except where otherwise noted, these bacterial strains can be used interchangeably. The genotypes listed are illustrative of many of the desired characteristics for choosing a bacterial host and are not meant to limit the

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invention in any way. The genotype designations are in accordance with standard nomenclature. See, for example, J. Sambrook, et al., supra. A preferred strain of E. coli employed in the cloning and expression of the genes of this 5 invention is RV308, which is available from the ATCC under accession number ATCC 31608, and is described in United States Patent 4,551,433, issued November 5, 1985.

In addition to the strains of E. coli discussed supra, bacilli such as Bacillus subtilis, other 10 enterobacteriaceae such as Salmonella typhimurium or Serratia marcescans, and various Pseudomonas species may be used. In addition to these gram-negative bacteria, other bacteria, especially Streptomyces, spp., may be employed in the prokaryotic cloning and expression of the proteins of this 15 invention.

Promoters suitable for use with prokaryotic hosts include the β -lactamase [vector pGX2907 (ATCC 39344) contains the replicon and β -lactamase gene] and lactose promoter systems [Chang et al., Nature (London), 275:615 (1978); and 20 Goeddel et al., Nature (London), 281:544 (1979)], alkaline phosphatase, the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695) is designed to facilitate expression of an open reading frame as a trpE fusion protein under control of the trp promoter] and hybrid promoters such as the tac promoter (isolatable from plasmid pDR540 ATCC-37282). However, other functional bacterial promoters, whose 25 nucleotide sequences are generally known, enable one of skill in the art to ligate them to DNA encoding the proteins of the instant invention using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial 30 systems also will contain a Shine-Dalgarno sequence operably linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized 35 either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or peptide which may be removable by

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enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion protein prolongs the lifespan, increases the yield of the desired peptide, or provides a 5 convenient means of purifying the protein of interest. A variety of peptidases (e.g. trypsin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (e.g. diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals 10 (e.g. cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal 15 cleavage sites. See e.g., P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13 in PROTEIN PURIFICATION: FROM MOLECULAR MECHANISMS TO LARGE SCALE PROCESSES, American Chemical Society, Washington, D.C. (1990).

In addition to cloning and expressing the genes of 20 interest in the prokaryotic systems discussed above, the proteins of the present invention may also be produced in eukaryotic systems. The present invention is not limited to use in a particular eukaryotic host cell. A variety of eukaryotic host cells are available from depositories such as 25 the American Type Culture Collection (ATCC) and are suitable for use with the vectors of the present invention. The choice of a particular host cell depends to some extent on the particular expression vector used to drive expression of the nucleic acids of the present invention. Exemplary host 30 cells suitable for use in the present invention are listed in Table I.

Table I

Host Cell	Origin	Source
HepG-2	Human Liver Hepatoblastoma	ATCC HB 8065

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CV-1	African Green Monkey Kidney	ATCC CCL 70
LLC-MK ₂	Rhesus Monkey Kidney	ATCC CCL 7.1
3T3	Mouse Embryo Fibroblasts	ATCC CCL 92
CHO-K1	Chinese Hamster Ovary	ATCC CCL 61
HeLa	Human Cervix Epitheloid	ATCC CCL 2
RPMI8226	Human Myeloma	ATCC CCL 155
H4IIEC3	Rat Hepatoma	ATCC CCL 1600
C127I	Mouse Fibroblast	ATCC CCL 1616
HS-Sultan	Human Plasma Cell Plasmacytoma	ATCC CCL 1484
<u>BHK-21</u>	<u>Baby Hamster Kidney</u>	<u>ATCC CCL 10</u>

An especially preferred cell line employed in this invention is the widely available cell line AV12-664 (hereinafter "AV12"). This cell line is available from the

5 American Type Culture Collection under the accession number ATCC CRL 9595. The AV12 cell line was constructed by injecting a Syrian hamster in the scruff of the neck with human adenovirus 12 and isolating cells from the resulting tumor.

10 A wide variety of vectors, some of which are discussed below, exists for the transformation of such mammalian host cells, but the specific vectors described herein are in no way intended to limit the scope of the present invention.

15 The pSV2-type vectors comprise segments of the simian virus 40 (SV40) genome that constitute a defined eukaryotic transcription unit-promoter, intervening sequence, and polyadenylation site. In the absence of the SV40 T antigen, the plasmid pSV2-type vectors transform mammalian

20 and other eukaryotic host cells by integrating into the host cell chromosomal DNA. A large number of plasmid pSV2-type vectors have been constructed, such as plasmid pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2-β-globin, in which the SV40 promoter drives transcription of an inserted gene.

25 These vectors are suitable for use with the coding sequences of the present invention and are widely available from

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sources such as the ATCC or the Northern Regional Research Laboratory (NRRL), 1815 N. University Street, Peoria, Illinois, 61604.

The plasmid pSV2-dhfr (ATCC 37146) comprises a murine dihydrofolate reductase (dhfr) gene under the control of the SV40 early promoter. Under the appropriate conditions, the dhfr gene is known to be amplified, or copied, in the host chromosome. This amplification can result in the amplification of closely-associated DNA sequences and can, therefore, be used to increase production of a protein of interest. See, e.g., J. Schimke, Cell, 35:705-713 (1984).

Plasmids constructed for expression of the proteins of the present invention in mammalian and other eukaryotic host cells can utilize a wide variety of promoters. The present invention is in no way limited to the use of the particular promoters exemplified herein. Promoters such as the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the gluco-corticoid-inducible tyrosine aminotransferase gene, and the thymidine kinase gene, and the major early and late adenovirus genes can be readily isolated and modified to express the genes of the present invention. Eukaryotic promoters can also be used in tandem to drive expression of a coding sequence of this invention. Furthermore, a large number of retroviruses are known that infect a wide range of eukaryotic host cells. The long terminal repeats in the retroviral DNA frequently encode functional promoters and, therefore, may be used to drive expression of the nucleic acids of the present invention.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. Gorman, et al., Proceedings of the National Academy of Sciences (USA), 79:6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises

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the long terminal repeats of the Murine Sarcoma virus, a virus known to infect mouse and other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the 5 expression of the nucleic acids of the present invention. The mouse metallothionein promoter is present in the plasmid pdBPV-MMTneo (ATCC 37224) which can serve as the starting material of other plasmids of the present invention.

An especially preferred expression vector system 10 employs one of a series of vectors containing the BK enhancer, an enhancer derived from the BK virus, a human papovavirus. The most preferred such vector systems are those which employ not only the BK enhancer but also the adenovirus-2-early region 1A (E1A) gene product. The E1A 15 gene product (actually, the E1A gene produces two products, which are collectively referred to herein as "the E1A gene product") is an immediate-early gene product of adenovirus, a large DNA virus.

A most preferred expression vector employed in the 20 present invention is the phd series of vectors which comprise a BK enhancer in tandem with the adenovirus late promoter to drive expression of useful products in eukaryotic host cells. The construction and method of using the phd plasmid, as well as related plasmids, are described in U.S. Patents 5,242,688, 25 issued September 7, 1993, and 4,992,373, issued February 12, 1991, as well as co-pending United States patent application 07/368,700, all of which are herein incorporated by reference. Escherichia coli K12 GM48 cells harboring the plasmid phd are available as part of the permanent stock 30 collection of the Northern Regional Research Laboratory under accession number NRRL B-18525. The plasmid may be isolated from this culture using standard techniques.

The plasmid phd contains a unique BclI site which 35 may be utilized for the insertion of the gene encoding the protein of interest. The skilled artisan understands that linkers or adapters may be employed in cloning the gene of interest into this BclI site. A depiction of the plasmid phd

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is provided as Figure 2 of this document. The phd series of plasmids functions most efficiently when introduced into a host cell which produces the E1A gene product, cell lines such as AV12-664, 293 cells, and others, described supra.

5 Transformation of the mammalian cells can be performed by any of the known processes including, but not limited to, the protoplast fusion method, the calcium phosphate co-precipitation method, electroporation and the like. See, e.g., J. Sambrook, et al., supra, at 3:16.30-
10 3:16.66.

Other routes of production are well known to skilled artisans. In addition to the plasmid discussed above, it is well known in the art that some viruses are also appropriate vectors. For example, the adenovirus, the adeno-
15 associated virus, the vaccinia virus, the herpes virus, the baculovirus, and the rous sarcoma virus are useful. Such a method is described in U.S. Patent 4,775,624, herein incorporated by reference. Several alternate methods of expression are described in J. Sambrook, et al., supra, at
20 16.3-17.44.

In addition to prokaryotes and mammalian host cells, eukaryotic microbes such as yeast cultures may also be used. The imperfect fungus Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used eukaryotic
25 microorganism, although a number of other strains are commonly available. For expression in Saccharomyces sp., the plasmid YRp7 (ATCC-40053), for example, is commonly used. See, e.g., L. Stinchcomb, et al., Nature, 282:39 (1979); J. Kingsman et al., Gene, 7:141 (1979); S. Tschemper et al.,
30 Gene, 10:157 (1980). This plasmid already contains the trp gene which provides a selectable marker for a mutant strain of yeast lacking the ability to grow in tryptophan.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase
35 [found on plasmid pAP12BD (ATCC 53231) and described in U.S. Patent No. 4,935,350, issued June 19, 1990, herein incorporated by reference] or other glycolytic enzymes such

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as enolase [found on plasmid pAC1 (ATCC 39532)], glyceraldehyde-3-phosphate dehydrogenase [derived from plasmid pHcGAPC1 (ATCC 57090, 57091)], hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate 5 isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase, as well as the alcohol dehydrogenase and pyruvate decarboxylase genes of Zymomonas mobilis (United States Patent No. 5,000,000 issued March 19, 1991, herein 10 incorporated by reference).

Other yeast promoters, which are inducible promoters, having the additional advantage of their transcription being controllable by varying growth conditions, are the promoter regions for alcohol 15 dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein [contained on plasmid vector pCL28XhoLHBPV (ATCC 39475) and described in United States Patent No. 4,840,896, herein incorporated by reference], glyceraldehyde 20 3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose [e.g. GAL1 found on plasmid pRY121 (ATCC 37658)] utilization. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman *et al.*, European Patent Publication No. 73,657A. 25 Yeast enhancers such as the UAS Gal from Saccharomyces cerevisiae (found in conjunction with the CYC1 promoter on plasmid YEpsec--hIibeta ATCC 67024), also are advantageously used with yeast promoters.

Practitioners of this invention realize that, in 30 addition to the above-mentioned expression systems, the cloned cDNA may also be employed in the production of transgenic animals in which a test mammal, usually a mouse, in which expression or overexpression of the proteins of the present invention can be assessed. The nucleic acids of the 35 present invention may also be employed in the construction of "knockout" animals in which the expression of the native cognate of the gene is suppressed.

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Skilled artisans also recognize that some alterations of SEQ ID NO:2 will fail to change the function of the amino acid compound. For instance, some hydrophobic amino acids may be exchanged for other hydrophobic amino acids. Those altered amino acid compounds which confer substantially the same function in substantially the same manner as the exemplified amino acid compound are also encompassed within the present invention. Typical such conservative substitutions attempt to preserve the: (a) secondary or tertiary structure of the polypeptide backbone; (b) the charge or hydrophobicity of the residue; or (c) the bulk of the side chain. Some examples of such conservative substitutions of amino acids, resulting in the production of proteins which are functional equivalents of the protein of SEQ ID NO:2 are shown in Table II, infra.

Table II

	Original Residue	Exemplary Substitutions
20	Ala	Ser, Gly
	Arg	Lys
	Asn	Gln, His
	Asp	Glu
	Cys	Ser
	Gln	Asn
25	Glu	Asp
	Gly	Pro, Ala
	His	Asn, Gln
	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
30	Met	Leu, Ile
	Phe	Met, Leu, Tyr
	Ser	Thr
	Thr	Ser
	Trp	Tyr

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Tyr	Trp, Phe
Val	Ile, Leu

These substitutions may be introduced into the protein in a
5 variety of ways, such as during the chemical synthesis or by
chemical modification of an amino acid side chain after the
protein has been prepared.

Alterations of the protein having a sequence which
corresponds to the sequence of SEQ ID NO:2 may also be
10 induced by alterations of the nucleic acid compounds which
encodes these proteins. These mutations of the nucleic acid
compound may be generated by either random mutagenesis
techniques, such as those techniques employing chemical
mutagens, or by site-specific mutagenesis employing
15 oligonucleotides. Those nucleic acid compounds which confer
substantially the same function in substantially the same
manner as the exemplified nucleic acid compounds are also
encompassed within the present invention.

Other embodiments of the present invention are
20 nucleic acid compounds which comprise isolated nucleic acid
sequences which encode SEQ ID NO:2. As skilled artisans will
recognize, the amino acid compounds of the invention can be
encoded by a multitude of different nucleic acid sequences
because most of the amino acids are encoded by more than one
25 nucleic acid triplet due to the degeneracy of the amino acid
code. Because these alternative nucleic acid sequences would
encode the same amino acid sequences, the present invention
further comprises these alternate nucleic acid sequences.

The gene encoding the hBNPI protein molecule may be
30 produced using synthetic methodology. This synthesis of
nucleic acids is well known in the art. See, e.g., E.L.
Brown, R. Belagaje, M.J. Ryan, and H.G. Khorana, Methods in
Enzymology, 68:109-151 (1979). The DNA segments
corresponding to the receptor gene are generated using
35 conventional DNA synthesizing apparatus such as the Applied
Biosystems Model 380A or 380B DNA synthesizers (commercially
available from Applied Biosystems, Inc., 850 Lincoln Center

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Drive, Foster City, CA 94404) which employ phosphoramidite chemistry. In the alternative, the more traditional phosphotriester chemistry may be employed to synthesize the nucleic acids of this invention. [See, e.g., M.J. Gait, ed.,

5 OLIGONUCLEOTIDE SYNTHESIS, A PRACTICAL APPROACH, (1984).]

The synthetic human hBNPI protein gene may be designed to possess restriction endonuclease cleavage sites at either end of the transcript to facilitate isolation from and integration into expression and amplification plasmids.

10 The choice of restriction sites are chosen so as to properly orient the coding sequence of the receptor with control sequences to achieve proper in-frame reading and expression of the hBNPI protein. A variety of other such cleavage sites may be incorporated depending on the particular plasmid
15 constructs employed and may be generated by techniques well known in the art.

In an alternative methodology, the desired DNA sequences can be generated using the polymerase chain reaction as described in U.S. Patent No. 4,889,818, which is 20 herein incorporated by reference.

In addition to the deoxyribonucleic acid of SEQ ID NO:1, this invention also provides ribonucleic acids (RNA) which comprise the RNA sequence

25	CGAUAGCUU GAUAUCGAAU UCCGGACUCU UGCUCGGCG CCUUAACCCG GCGUUCGGUU	60
	CAUCCCGCAG CGCCAGUUCU GCUUACCAAA AGUGGCCAC UAGGCACUCG CAUUCCACGC	120
	CCGGCUCCAC GCCAGCGAGC CGGGCUUCUU ACCCAUUJAA AGUUUJAGAGAA UAGGUUGAGA	180
30	UCGUUUCGGC CCCAAGACCU CUAAUCAUJC GCUUUACCGG AUAAAACUGC GUGGCGGGGG	240
	UGCGUCGGGU CUGCGAGAGC GCCAGCUAUC CUGAGGGAAA CUUCGGAGGG AACCAAGCUAC	300
	35 UAGAUGGUUC GAUUAGUCUU UCGCCCCUAU ACCCAGGUCG GACGACCGAU UUGCACGUCA	360
	GGACCGCUAC GGACCUCCAC CAGAGUUUCC UCUGGCUUCG CCCUGCCCAG GCGAUCGGCG	420
	GGGGGGACCC GCGGGGUGAC CGGCGGCAGG AGCCGCCACC AUGGAGUUCC GCCAGGAGGA	480
40	GUUUCGGAAG CUAGCGGGUC GUGGCUCUCGG GAAGCUGCAC CGCCUUCUGG AGAACGGCA	540
	GGAAGGCGCG GAGACCGUGG AGCUGAGUGC GGAUGGGCGC CCGGUGACCA CGCAGACCCG	600

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	GGACCCGCCG GUGGUGGACU GCACCUGCUU CGGCCUCCCU CGCCGCUACA UUAUCGCCAU	660
5	CAUGAGUGGU CUGGGCUUCU GCAUCAGCUU UGGCAUCCGC UGCAACCUGG GCGUGGCCAU	720
	CGUCUCCAUG GUCAAUAACA GCACGACCCA CCCGGGGGC CACGUGGUGG UGCAGAAAGC	780
	CCAGUUCAGC UGGGAUCCAG AGACUGUCGG CCUCAUACAC GGCUCCUUUU UCUGGGGCUA	840
10	CAUUGUCACU CAGAUUCCAG GAGGAUUUAU CUGUAAAAA UUUGCAGCCA ACAGAGUUUU	900
	CGGCUUUGCU AUUGUGGCAA CAUCCACUCU AAACAUGCUG AUCCCCUCAG CUGCCCGCGU	960
15	CCACUAUGGC UGUGUCAUCU UCGUGAGGAU CCUGCAGGGG UUGGUAGAGG GGGUCACAU	1020
	CCCCGCCUGC CAUGGGAUCU GGAGCAAAUG GGCCCCACCC UUAGAACGGA GUCGCCUGGC	1080
	GACGACAGCC UUUUGUGGUU CCUAUGCUGG GGC GGUGGUC GCGAUGCCCC UCGCCGGGU	1140
20	CCUUGUGCAG UACUCAGGAU GGAGCUCUGU UUUUCUACGUC UACGGCAGCU UCGGGGAUCUU	1200
	CUGGUACCUG UUCUGGCUGC UCGUCUCCUA CGAGUCCCCC GCGCUGCACC CCAGCAUCUC	1260
25	GGAGGAGGAG CGCAAGUACA UCGAGGACCC CAUCGGAGAG AGCGCGAAC UCAUGAACCC	1320
	CCUCACGAAG UUUAGCACUC CCUGGCGGGCG CUUCUUCACG UCUAUGCCAG UCUAUGCCAU	1380
	CAUCGUGGCC AACUUCUGCC GCAGCUGGAC GUUCUACCUG CUGCUCAUCU CCCAGCCCC	1440
30	CUACUUCGAA GAAGUGUUCG GCUUCGAGAU CAGCAAGGUA GCCCUGGUGU CCGCGCUGCC	1500
	CCACCUUGGUC AUGACCAUCA UCGUGCCCAU CGGCGGCCAG AUCGGGACU UCCUGGGAG	1560
35	CCGCCGCAUC AUGUCCACCA CCAACGUGCG CAAGUUGAUG AACUGCGGAG GCUUCGGCAU	1620
	GGAAGCCACG CUGCUGUJGG UGGUCGGCUA CUCGCACUCC AAGGGCGUGG CCAUCUCCUU	1680
	CCUGGUCCUA GCCGUGGGCU UCAGCGGCUU CGCCAUCUCU GGGUCAACG UGAACCACCU	1740
40	GGACAUAGCC CCGCGCUACG CCAGCAUCCU CAUGGGCAUC UCCAACGGCG UGGGCACACU	1800
	GUCGGGCAUG GUGUGCCCCA UCAUCGUGGG GGCCAUGACU AAGCACAAGA CUCGGGAGGA	1860
45	GUGGCAGUAC GUGUUCCUAA UUGCCUCCCU GGUGGCACUAA GGAGGUGUCA UCUUCUACGG	1920
	GGUCUUUGCU UCUGGAGAGA ACCAGCGUG GGCAGAGCCU GAGGAGAUGA GCGAGGAGAA	1980
	GUGUGGCCUUC GUUGGCCAUG ACCAGCUGGC UGGCAGUGAC GACAGCGAAA UGGAGGAUGA	2040
50	GGCUGAGCCC CCGGGGGCAC CCCCUGCACC CCCGCCUCC UAUGGGGCCA CACACAGCAC	2100
	AUUCAGCCCC CCCAGGGCCCC CACCCCCUGU CGGGACUAC UGACCAUGUG CCUCCCACUG	2160
55	AAUGGCAGUU UCCAGGACCU CCAUUCCACU CAUCUCUGGC CUGAGUGACA GUGUCAAGGA	2220

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	ACCCUGCUCC UCUCUGUCCU GCCUCAGGCC UAAGAAGCAC UCUCCCUUGU UCCCAGUGCU	2280
	GUCAAAUCCU CUUUCCUUCC CAAUUGCUC UCAGGGGUAG UGAAGCUGCA GACUGACAGU	2340
5	UUCAAGGAUA CCCAAAUCC CCUAAAGGUU CCCUCUCCAC CGGUUCUGCC UCAGUGGUUU	2400
	CAAUAUCUCU CUUUCAGGGC UUUAUUUGAA UGGACAGUUC GACCUCUUAC UCUCUCUUGU	2460
10	GGUUUUGAGG CACCCACACC CCCCCGUUUC CUUUAUCUCC AGGGACUCUC AGGCUAACCU	2520
	UUGAGAUCAC UCAGCUCCCA UCUCCUUUCA GAAAAAUUCA AGGUCCUCCU CUAGAAGUUU	2580
	CAAUAUCUCU CCAACUCUGU UCUGCAUCUU CCAGAUUGGU UUAACCAAUU ACUCGUCCCC	2640
15	GCCAUUCCAG GGAAUUGAUUC UCACCAGCGU UUCUGAUGGA AAAUGGCGGG AAUUCCUGCA	2700
	GCCCCGGGGGA UCCACU	2716

hereinafter referred to as SEQ ID NO:3, or the complementary
20 ribonucleic acid, or a fragment of either SEQ ID NO:3 or the complement thereof. The ribonucleic acids of the present invention may be prepared using the polynucleotide synthetic methods discussed supra or they may be prepared enzymatically using RNA polymerases to transcribe a DNA template.

25 complement thereof.

The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. Both of these RNA polymerases are highly specific and require the insertion of bacteriophage-specific sequences at 30 the 5' end of the message to be read. See, J. Sambrook, et al., supra, at 18.82-18.84.

This invention also provides nucleic acids, RNA or DNA, which are complementary to SEQ ID NO:1 or SEQ ID NO:3.

35 The present invention also provides probes and primers useful for molecular biology techniques. A compound which encodes for SEQ ID NO:1, SEQ ID NO:3 or a complementary sequence of SEQ ID NO:1 or SEQ ID NO:3, or a fragment thereof, and which is at least 18 base pairs in length, and 40 which will selectively hybridize to human genomic DNA or messenger RNA encoding a human brain Na⁺-dependent inorganic

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phosphate cotransporter, is provided. Preferably, the 18 or more base pair compound is DNA.

The term "selectively hybridize" as used herein may refer to either of two situations. In the first such embodiment of this invention, the nucleic acid compounds described supra hybridize to a human sodium-dependent inorganic phosphate cotransporter under more stringent hybridization conditions than these same nucleic acid compounds would hybridize to an analogous sodium-dependent inorganic phosphate cotransporter of another species, e.g. murine or primate. In the second such embodiment of this invention, these probes hybridize to the hBNPI protein of the present invention under more stringent hybridization conditions than other related compounds, including nucleic acid sequences encoding other ion cotransporters.

These probes and primers can be prepared enzymatically as described supra. In a most preferred embodiment these probes and primers are synthesized using chemical means as described supra. Probes and primers of defined structure may also be purchased commercially.

This invention also encompasses recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. Many of the vectors encompassed within this invention are described above. The preferred nucleic acid vectors are those which are DNA.

The sequence of SEQ ID NO:1 was prepared as follows:

Molecular cloning of a human brain Na⁺-dependent inorganic phosphate cotransporter (hBNPI)

Using a cDNA encoding the rat brain Na⁺-dependent inorganic phosphate cotransporter (rBNPI) (Ni, et al., 1994), we screened, under low stringency conditions, a human cDNA library derived from hippocampus mRNAs. Twelve positive clones were isolated that strongly hybridized to the ³²P-labeled probe rBNPI. Restriction endonuclease analysis

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and/or sequencing of these clones revealed two distinct sequences: those which are highly similar to the rBNPI (B. Ni, *et al.*, 1994, *supra*) as well as the kidney Na⁺-dependent inorganic phosphate cotransporter (Na/Pi), found in 10
5 clones, and those found in 2 clones which were proved to be rearrangements between the human putative phosphate transporter and other cDNAs. Of the 10 clones (designed as hBNP) which exhibited a strong similarity to rBNPI, 4 clones contained the 2.7 kb message. Sequence analysis of hBNPI
10 predicts an open reading frame of 1683 bases, corresponding to a protein of 560 amino acids with an apparent molecular mass of 61,000 Da (61 kDa). The ATG initiation codon at position 1, which is preceded by an upstream, in-frame stop codon, matches the Kazak consensus initiation sequence for
15 the initiation of translation.

Computer searching revealed that the protein encoded by the hBNPI shared significant sequence homology at the amino acid level with those of recently cloned rat rBNPI (98%), rabbit (31%) and human (31%) kidney phosphate
20 transporter, Na/Pi, as indicated by comparison analysis. The highest degree of homology, which was found between rBNPI and hBNPI, suggested that hBNPI is the human homologue of the rat rBNPI. The segment of highest homology among the proteins is confined to a region that fits the proposed
25 consensus Na⁺-binding domain for various Na⁺-dependent transporter systems (Deguchi *et al.*, 1990). Alignment of the predicted hBNPI protein sequence with the consensus sequence indicated that amino acids leucine (L), glycine (G) and arginine (R) residues match the proposed motif and that other
30 (F and R) are conservatively changed. The predicted hBNPI protein sequence also shares 41% and 32% amino acid identity with two proteins of unknown function from *Caenorhabditis elegans*, ZK512.6 and C38C10.2, respectively. J. Sulston *et al.*, Nature (London), 356:37-41 (1992). A hydropathy plot of
35 the deduced amino acid sequence of hBNPI suggests the presence of at least 6 to 8 transmembrane regions. This

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number of membrane-spanning domains is a characteristic structural motif of transport proteins. Based on the convention that activity of neuronal Pi transport correlates with ATP synthesis and intracellular energy charge, we have
5 modelled hBNPI protein secondary structure with 6 transmembrane domains, which is consistent with those of other energy-linked anion transporters. The putative two glycosylation sites and two protein kinase C phosphorylation sites and four putative calmodulin-dependent kinase II
10 phosphorylation sites are well conserved

The skilled artisan understands that the type of cloning vector or expression vector employed depends upon the availability of appropriate restriction sites, the type of host cell in which the vector is to be transfected or
15 transformed, the purpose of the transfection or transformation (e.g., transient expression in an oocyte system, stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable markers (e.g., antibiotic
20 resistance markers, metabolic markers, or the like), and the number of copies of the gene to be present in the cell.

The type of vector employed to carry the nucleic acids of the present invention may be RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages,
25 stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors of the present invention are those derived from plasmids.

When preparing an expression vector the skilled artisan understands that there are many variables to be
30 considered. One such example is the use of a constitutive promoter, i.e. a promoter which is functional at all times, instead of a regulatable promoter which may be activated or inactivated by the artisan using heat, addition or removal of a nutrient, addition of an antibiotic, and the like. The
35 practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. For experiments examining the amount

of the protein expressed on the cell membrane or for experiments examining the biological function of an expressed membrane protein, for example, it may be unwise to employ an expression system which produces too much of the protein.

5 The addition or subtraction of certain sequences, such as a signal sequence preceding the coding sequence, may be employed by the practitioner to influence localization of the resulting polypeptide. Such sequences added to or removed from the nucleic acid compounds of the present invention are
10 encompassed within this invention.

The starting plasmids employed to prepare the vectors of the present invention may be isolated from the appropriate E. coli containing these plasmids using standard procedures such as cesium chloride DNA isolation.

15 The plasmids of the present invention may be readily modified to construct expression vectors that produce hBNPI proteins in a variety of organisms, including, for example, E. coli, Sf9 (as host for baculovirus), Spodoptera and Saccharomyces. The current literature contains
20 techniques for constructing AV12 expression vectors and for transforming AV12 host cells. United States Patent No. 4,992,373, herein incorporated by reference, is one of many references describing these techniques.

One of the most widely employed techniques for
25 altering a nucleic acid sequence is by way of oligonucleotide-directed site-specific mutagenesis. B. Comack, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 8.01-8.5.9, (F. Ausubel, et al., eds. 1991). In this technique an oligonucleotide, whose sequence contains the mutation of
30 interest, is synthesized as described supra. This oligonucleotide is then hybridized to a template containing the wild-type sequence. In a most preferred embodiment of this technique, the template is a single-stranded template. Particularly preferred are plasmids which contain regions
35 such as the f1 intergenic region. This region allows the generation of single-stranded templates when a helper phage is added to the culture harboring the "phagemid".

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After the annealing of the oligonucleotide to the template, a DNA-dependent DNA polymerase is then used to synthesize the second strand from the oligonucleotide, complementary to the template DNA. The resulting product is 5 a heteroduplex molecule containing a mismatch due to the mutation in the oligonucleotide. After DNA replication by the host cell a mixture of two types of plasmid are present, the wild-type and the newly constructed mutant. This technique permits the introduction of convenient restriction 10 sites such that the coding sequence may be placed immediately adjacent to whichever transcriptional or translational regulatory elements are employed by the practitioner.

The construction protocols utilized for E. coli can be followed to construct analogous vectors for other 15 organisms, merely by substituting, if necessary, the appropriate regulatory elements using techniques well known to skilled artisans.

Host cells which harbor the nucleic acids provided by the present invention are also provided. A preferred host 20 cell is an Xenopus sp. oocyte which has been injected with RNA or DNA compounds of the present invention. Most preferred oocytes of the present invention are those which harbor a sense mRNA of the present invention. Other preferred host cells include AV12 and E. coli cells which 25 have been transfected and/or transformed with a vector which comprises a nucleic acid of the present invention.

The present invention also provides a method for constructing a recombinant host cell capable of expressing SEQ ID NO:2, said method comprising transforming a host cell 30 with a recombinant DNA vector that comprises an isolated DNA sequence which encodes SEQ ID NO:2. The preferred host cell is AV12. The preferred vector for expression is one which comprises SEQ ID NO:1. Another preferred host cell for this method is E. coli. An especially preferred expression vector 35 in E. coli is one which comprises SEQ ID NO:1. Transformed host cells may be cultured under conditions well known to

skilled artisans such that SEQ ID NO:2 is expressed, thereby producing Yb in the recombinant host cell.

The ability of ions to bind to the hBNPI protein is essential in the development of a multitude of indications.

5 In developing agents which act as antagonists or agonists of the hBNPI protein, it would be desirable, therefore, to determine those agents which bind the hBNPI protein.
Generally, such an assay includes a method for determining whether a substance is a functional ligand of the hBNPI
10 protein, said method comprising contacting a functional compound of the hBNPI protein with said substance, monitoring binding activity by physically detectable means, and identifying those substances which effect a chosen response. Preferably, the physically detectable means is competition
15 with labeled inorganic phosphate or binding of ligand in an oocyte transient expression system

The instant invention provides such a screening system useful for discovering agents which compete with inorganic phosphate for binding to the hBNPI protein, said
20 screening system comprising the steps of:

- a) isolating a human hBNPI protein;
- b) exposing said human hBNPI protein to a potential inhibitor or surrogate of the Pi/hBNPI protein complex;
- c) introducing Pi;
- d) removing non-specifically bound molecules; and
- e) quantifying the concentration of bound potential inhibitor and/or Pi.

30 This allows one to rapidly screen for inhibitors or surrogates of the formation of the Pi/hBNPI protein complex. Utilization of the screening system described above provides a sensitive and rapid means to determine compounds which

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interfere with the formation of the Pi/hBNPI protein complex. This screening system may also be adapted to automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system allowing for efficient high-volume screening of potential
5 therapeutic agents.

In such a screening protocol a hBNPI protein is prepared as elsewhere described herein, preferably using recombinant DNA technology. A sample of a test compound is then introduced to the reaction vessel containing the hBNPI
10 protein followed by the addition of Pi. In the alternative the Pi may be added simultaneously with the test compound. Unbound molecules are washed free and the eluent inspected for the presence of Pi or the test compound.

For example, in a preferred method of the
15 invention, radioactively labeled Pi may be used. The eluent is then scored for the radioactivity. The absence or diminution of the chemical label or radioactivity indicates the formation of the Pi/hBNPI protein complex. This indicates that the test compound has not effectively competed
20 with Pi in the formation of the Pi/hBNPI protein complex. The presence of the chemical label or radioactivity indicates that the test compound has competed with Pi in the formation of the Pi/hBNPI protein complex. Similarly, a radioactively or chemically labeled test compound may be used in which case
25 the same steps as outlined above would be used except that the interpretation of results would be the converse of using radioactively labelled Pi.

As would be understood by the skilled artisan these assays may also be performed such that the practitioner
30 measures the radioactivity remaining with the protein, not in the eluent. A preferred such assay employs radiolabeled Pi. After the competition reaction has been performed the reaction mixture is then passed through a filter, the filter retaining the receptor and whatever is complexed with the
35 receptor. The radioactivity on each filter is then measured in a scintillation counter. In such an assay higher amounts

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of radiolabel present indicate lower affinity for the receptor by the test compound.

The hBNPI protein may be free in solution or bound to a solid support. Whether the hBNPI protein is bound to a support or is free in solution, it is generally important that the conformation of the protein be conserved. In a preferred practice of the invention, therefore, the hBNPI protein is suspended in a hydrophobic environment employing natural or synthetic detergents, membrane suspensions, and the like. Preferred detergent complexes include the zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate ("CHAPS") as well as sodium deoxycholate.

Skilled artisans will recognize that desirable dissociation constant (K_i) values are dependent on the selectivity of the compound tested. For example, a compound with a K_i which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound which has a lower affinity, but is selective for the particular receptor, may be an even better candidate. The present invention, however, provides radiolabeled competition assays, whether results therefrom indicate high affinity or low affinity to hBNPI protein, because skilled artisans will recognize that any information regarding binding or selectivity of a particular compound is beneficial in the pharmaceutical development of drugs.

Assays useful for evaluating ion channel cotransporters are well known in the art. See, e.g., B. Ni, et al., supra. One such assay is described below.

Functional analysis of hBNPI in transfected COS-1 cells

To confirm the functional properties of the hBNPI protein, we constructed the hBNPI cDNA into a mammalian expression vector (pcDNA3) and transfected the pcDNA3-hBNPI constructs into the COS-1 cells. Sodium-dependent ^{32}Pi uptake

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in the cells transfected with hBNPI was stimulated 2-3 fold above that of those transfected with vectors alone or of nontransfected cells. Replacement of sodium chloride with choline chloride reduced ^{32}Pi uptake to background levels.

5 Northern blot analysis was employed to examine the expression of hBNPI gene in transfected COS-1 cell lines. Labeled hBNPI cDNA detected strong expression of hBNPI transcripts in the COS-1 cells transfected with hBNPI but not in those cells transfected with the vector alone.

10

Expression of hBNPI mRNA in human brain

We examined hBNPI expression in multiple human tissues by probing polyadenylated RNA from heart, brain, 15 placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes. The Northern blot analysis demonstrated that hBNPI probe detected a single mRNA species of 2.8 kb and strong expression of hBNPI transcript 20 in the brain tissue. Trace levels of the hBNPI could be detected in RNA fractions from the small intestine, colon and testis if the blot was overexposed for a longer period of time (five days versus the usual one day exposure). No signal could be detected in the other tissues. The level of 25 hBNPI in the brain fraction is at least 100 times higher than that in the intestine or colon. Northern blot analysis with multiple human brain regions shows that hBNPI mRNA is expressed in specific brain regions: most abundantly in neuron-enriched areas such as the amygdala and hippocampus; 30 at moderate levels in glia-enriched areas such as the corpus callosum; and at low levels in the substantia nigra, subthalamic nuclei and thalamus. No hBNPI transcript was detected in RNAs isolated from the caudate nucleus and hypothalamus.

35 A Northern blot of human brain mRNA isolated from fetal and adult (37 yr-old) brain was prepared for the characterization of expression of the hBNPI during brain

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development. The blot was hybridized with ^{32}P -labeled hBNPI cDNA and human β -actin cDNA. The relative abundance of hBNPI mRNA shows a dramatic increase during postnatal development.

In situ hybridization histochemistry was employed
5 to examine cells which express hBNPI transcripts in the human brain. hBNPI mRNA is highly expressed in the hippocampus formation and cerebral cortex. While the hybridization signal is present in various layers of the cerebral cortex, it appears to be more abundant in the neuronal layer v-vi
10 where a distinct labeling is observed of pyramidal and non-pyramidal neurons. On closer inspection, it is apparent that hBNPI transcripts are concentrated in the pyramidal neurons of hippocampus and granule neurons of dentate gyrus. The hybridization signal was also detected in glia-enriched areas
15 such as the corpus callosum, a finding which is consistent with data observed in Northern blot analysis of hBNPI mRNA in the human brain, and which suggests that, unlike its rat counterpart rBNPI, the hBNPI mRNA is expressed not only in neurons but also in glia as well. Cf., Ni, et al., supra.
20

Genomic analysis of the hBNPI gene

Genomic Southern blotting is a valuable tool for identifying homologous genes in various species. We used
25 hBNPI cDNA to detect hBNPI genes in a variety of vertebrate species under stringent hybridization condition. The species tested included human, monkey, rat, mouse, dog, cow, rabbit, chicken and yeast. One major fragment which appears to harbor hBNPI gene was detected in the human, monkey, dog, cow
30 and rabbit. Two fragments generated by internal EcoRI sites were detected in the rat and mouse. No signal was detected in yeast DNA. The results suggest that hBNPI sequence is well conserved among vertebrate species.

Genomic DNAs derived from four human individuals
35 were digested with restriction endonucleases and used to determine the hBNPI gene structure and possible polymorphisms by Southern blot techniques utilizing the full length hBNPI

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cDNA as a probe. The restriction patterns derived from 9 restrictions endonucleases are rather simple, and are similar between the four individuals. One major hybridizing fragment is generated by internal EcoRI, BalII, HindIII, PstI, PvuII, 5 respectively. One major fragment with multiple weak hybridizing bands was generated by internal digestion with TaqI, MspI and BamHI. The results suggest that hBNPI gene structure is compact, that it is most likely present as a single copy, and that no polymorphisms of hBNPI gene exist.

10

Chromosome localization

Using hBNPI cDNA we screened a library constructed with human leukocyte DNA to isolate the hBNPI gene. After 15 several rounds of screening, a 23 kb DNA fragment was isolated and identified as hBNPI gene. The hBNPI gene was labeled with digoxigenin dUTP by nick translation and hybridized to normal metaphase chromosomes derived from PHA-stimulated peripheral blood lymphocytes using a fluorescent 20 in situ hybridization (FISH) technique. A specific hybridization signal was detected in the long arm of chromosome 19. Assignment of the hBNPI gene to the region of 19 was further confirmed by colocalization of a chromosome 19 specific probe, E2A, with the hBNPI gene. Measurements of 25 ten specifically hybridized chromosomes 19 demonstrated that hBNPI gene is located 66% of the distance from the centromere to the telomere of chromosome arm 19q, an area that corresponds to band 19q13.3. No positive signals were observed in any other chromosomes. Analysis of interphase 30 cells show only one copy of the probe present in the human genome, a finding which is consistent with the results of the genomic Southern blot.

The previously described screening systems identify 35 compounds which competitively bind to the hBNPI protein. Determination of the ability of such compounds to stimulate or inhibit the action of the hBNPI protein is essential to

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further development of such compounds for therapeutic applications. The need for a bioactivity assay system which determines the response of the hBNPI protein to a compound is clear. The instant invention provides such a bioactivity 5 assay, said assay comprising the steps of:

10

- a) transfected a mammalian host cell with an expression vector comprising DNA encoding a hBNPI protein;
- b) culturing said host cell under conditions such that the DNA encoding the hBNPI protein is expressed,
- c) exposing said host cell so transfected to a test compound, and
- d) measuring the change in a physiological condition known to be influenced by the binding of a cation to the hBNPI protein relative to a control in which 20 the transfected host cell is not exposed to the test compound.

15
20

An oocyte transient expression system can be constructed according to the procedure described in S. 25 Lübbert, et al., Proceedings of the National Academy of Sciences (USA), 84:4332 (1987).

30

In an especially preferred embodiment of this invention an assay measuring the inhibition of radiolabeled phosphate uptake was performed. The inhibition of phosphate uptake is a relatively simple assay used to determine those agents which negatively affect the proteins of the present invention.

35

In another embodiment this invention provides a

method for identifying, in a test sample, DNA homologous to a probe of the present invention, wherein the test nucleic acid is contacted with the probe under hybridizing conditions and

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identified as being homologous to the probe. Hybridization techniques are well known in the art. See, e.g., J. Sambrook, et al., supra, at Chapter 11.

The nucleic acid compounds of the present invention
5 may also be used to hybridize to genomic DNA which has been digested with one or more restriction enzymes and run on an electrophoretic gel. The hybridization of radiolabeled probes onto such restricted DNA, usually fixed to a membrane after electrophoresis, is well known in the art. See, e.g.,
10 J. Sambrook, supra. Such procedures may be employed in searching for persons with mutations in these receptors by the well-known techniques of restriction fragment length polymorphisms (RFLP), the procedures of which are described in U.S. Patent 4,666,828, issued May 19, 1987, the entire
15 contents of which is herein incorporated by reference.

The proteins of this invention as well as fragments of these proteins may be used as antigens for the synthesis of antibodies. The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not
20 limited, to Fab, Fab', Fab₂', and Fv fragments), and chimeric, humanized, veneered, resurfaced, or CDR-grafted antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived. The instant invention also encompasses single chain
25 polypeptide binding molecules.

The term "antibody" as used herein is not limited by the manner in which the antibodies are produced, whether such production is in situ or not. The term "antibody" as used in this specification encompasses those antibodies
30 produced by recombinant DNA technology means including, but not limited, to expression in bacteria, yeast, insect cell lines, or mammalian cell lines.

The production of antibodies, both monoclonal and polyclonal, in animals, especially mice, is well known in the
35 art. See, e.g., C. Milstein, HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, (Blackwell Scientific Pub., 1986); J. Goding, Monoclonal ANTIBODIES: PRINCIPLES AND PRACTICE, (Academic Press, 1983). For

the production of monoclonal antibodies the basic process begins with injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells, resulting 5 in a hybridoma that reproduces in vitro. The population of hybridomas is screened to isolate individual clones, each of which secretes a single antibody species, specific for the immunogen. The individual antibody species obtained in this way is each the product of a single B cell from the immune 10 animal generated in response to a specific antigenic site, or epitope, recognized on the immunogenic substance.

Chimeric antibodies are described in U.S. Patent No. 4,816,567, which issued March 28, 1989 to S. Cabilly, et al. This reference discloses methods and vectors for the 15 preparation of chimeric antibodies. The entire contents of U.S. Patent No. 4,816,567 are herein incorporated by reference. An alternative approach to production of genetically engineered antibodies is provided in U.S. Patent No. 4,816,397, which also issued March 28, 1989 to M. Boss, 20 et al., the entire contents of which are herein incorporated by reference. The Boss patent teaches the simultaneous co-expression of the heavy and light chains of the antibody in the same host cell.

The approach of U.S. Patent 4,816,397 has been 25 further refined as taught in European Patent Publication No. 0 239 400, which published September 30, 1987. The teachings of this European patent publication (Winter) are a preferred format for the genetic engineering of the reactive monoclonal antibodies of this invention. The Winter technology involves 30 the replacement of complementarity determining regions (CDRs) of a human antibody with the CDRs of a murine monoclonal antibody thereby converting the specificity of the human antibody to the specificity of the murine antibody which was the source of the CDR regions. This "CDR grafting" 35 technology affords a molecule containing minimal murine sequence and thus is less immunogenic.

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Single chain antibody technology is yet another variety of genetically engineered antibody which is now well known in the art. See, e.g. R.E. Bird, et al., Science 242:423-426 (1988); Patent Cooperation Treaty Publication No. 5 WO 88/01649, which was published 10 March 1988. The single chain antibody technology involves joining the binding regions of heavy and light chains with a polypeptide sequence to generate a single polypeptide having the binding specificity of the antibody from which it was derived.

10 The aforementioned genetic engineering approaches provide the skilled artisan with numerous means to generate molecules which retain the binding characteristics of the parental antibody while affording a less immunogenic format.

15 These antibodies are used in diagnostics, therapeutics or in diagnostic/therapeutic combinations. By "diagnostics" as used herein is meant testing that is related to either the in vitro or in vivo diagnosis of disease states or biological status in mammals, preferably in humans. By "therapeutics" and "therapeutic/diagnostic combinations" as 20 used herein is respectively meant the treatment or the diagnosis and treatment of disease states or biological status by the in vivo administration to mammals, preferably humans, of the antibodies of the present invention. The antibodies of the present invention are especially preferred 25 in the diagnosis and/or treatment of conditions associated with an excess or deficiency of hBNPI proteins.

In addition to being functional as direct therapeutic and diagnostic aids, the availability of a family 30 of antibodies which are specific for the hBNPI protein enables the development of numerous assay systems for detecting agents which bind to this protein. One such assay system comprises radiolabeling hBNPI protein-specific antibodies with a radionuclide such as ¹²⁵I and measuring displacement of the radiolabeled hBNPI protein-specific 35 antibody from solid phase hBNPI protein in the presence of a potential antagonist or inhibitor.

Numerous other assay systems are also readily adaptable to detect agents which bind hBNPI protein. Examples of these aforementioned assay systems are discussed in Methods in Enzymology, (J. Langone, and H. Vunakis, eds.

5 1981), Vol. 73, Part B, the contents of which are herein incorporated by reference. Skilled artisans are directed to Section II of Methods in Enzymology, Vol. 73, Part B, supra, which discusses labeling of antibodies and antigens, and Section IV, which discusses immunoassay methods.

10 In addition to the aforementioned antibodies specific for the hBNPI protein, this invention also provides antibodies which are specific for the hypervariable regions of the anti-hBNPI protein antibodies. Some such anti-idiotypic antibodies would resemble the original epitope, the
15 hBNPI protein, and, therefore, would be useful in evaluating the effectiveness of compounds which are potential antagonists, agonists, or partial agonists of the hBNPI protein. See, e.g., Cleveland, et al., Nature (London), 305:56 (1983); Wasserman, et al., Proceedings of the National
20 Academy of Sciences (USA), 79:4810 (1982).

In another embodiment, this invention encompasses pharmaceutical formulations for parenteral administration which contain, as the active ingredient, the anti-hBNPI protein antibodies described, supra. Such formulations are
25 prepared by methods commonly used in pharmaceutical chemistry.

Products for parenteral administration are often formulated and distributed in solid, preferably freeze-dried form, for reconstitution immediately before use. Such
30 formulations are useful compositions of the present invention. Their preparation is well understood by pharmaceutical chemists.

In general, these formulations comprise the active ingredient in combination with a mixture of inorganic salts,
35 to confer isotonicity, as well as dispersing agents such as lactose, to allow the dried preparation to dissolve quickly

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upon reconstitution. Such formulations are reconstituted for use with highly purified water to a known concentration.

Alternatively, a water soluble form of the antibody can be dissolved in one of the commonly used intravenous 5 fluids and administered by infusion. Such fluids include physiological saline, Ringer's solution or a 5% dextrose solution.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Ni, Binhui
Paul, Steven M.
- (ii) TITLE OF INVENTION: HUMAN BRAIN SODIUM DEPENDENT INORGANIC PHOSPHATE COTRANSporter AND RELATED NUCLEIC ACID COMPOUNDS
- (iii) NUMBER OF SEQUENCES: 3
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Eli Lilly and Company
 - (B) STREET: Lilly Corporate Center
 - (C) CITY: Indianapolis
 - (D) STATE: Indiana
 - (E) COUNTRY: United States of America
 - (F) ZIP: 46285
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/430,033
 - (B) FILING DATE: April 27, 1995
 - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Blalock, Donna K.
 - (B) REGISTRATION NUMBER: 38,082
 - (C) REFERENCE/DOCKET NUMBER: X-10006
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (317) 276-0756
 - (B) TELEFAX: (317) 276-3861

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2716 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 461..2143

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGATAAGCTT GATATCGAAT TCCGGACTCT TGCTCGGGCG CCTTAACCCG GCGTCGGTT 60
 CATCCCGCAG CGCCAGTTCT GCTTACCAAA AGTGGCCCAC TAGGCACTCG CATTCCACGC 120
 CCGGCTCCAC GCCAGCGAGC CGGGCTTCTT ACCCATTTAA AGTTTGAGAA TAGGTTGAGA 180
 TCGTTTCGGC CCCAAGACCT CTAATCATTG GCTTTACCGG ATAAAACGTG GTGGCGGGGG 240
 TGCCTCGGGT CTGCGAGAGC GCCAGCTATC CTGAGGGAAA CTTCCGAGGG AACCAGCTAC 300
 TAGATGGTTC GATTAGTCTT TCGCCCCAT ACCCAGGTG GACGACCGAT TTGCACGTCA 360
 GGACCGCTAC GGACCTCCAC CAGAGTTTCC TCTGGCTTCG CCCTGCCAG GCGATCGGCG 420
 GGGGGGACCC CGGGGGTGAC CGGCAGCAGG AGCCGCCACC ATG GAG TTC CGC CAG 475
 Met Glu Phe Arg Gln
 1 5
 GAG GAG TTT CCG AAG CTA GCG GGT CGT GCT CTC GGG AAG CTG CAC CGC 523
 Glu Glu Phe Arg Lys Leu Ala Gly Arg Ala Leu Gly Lys Leu His Arg
 10 15 20
 CTT CTG GAG AAG CGG CAG GAA GGC GCG GAG ACG CTC GAG CTG AGT GCG 571
 Leu Leu Glu Lys Arg Gln Glu Gly Ala Glu Thr Leu Glu Leu Ser Ala
 25 30 35
 GAT GGG CGC CCG GTG ACC ACG CAG ACC CGG GAC CCG CCG GTG GTG GAC 619
 Asp Gly Arg Pro Val Thr Thr Gln Thr Arg Asp Pro Pro Val Val Asp
 40 45 50
 TGC ACC TGC TTC GGC CTC CCT CGC CGC TAC ATT ATC GCC ATC ATG AGT 667
 Cys Thr Cys Phe Gly Leu Pro Arg Arg Tyr Ile Ile Ala Ile Met Ser
 55 60 65
 GGT CTG GGC TTC TGC ATC AGC TTT GGC ATC CGC TGC AAC CTG GGC GTG 715
 Gly Leu Gly Phe Cys Ile Ser Phe Gly Ile Arg Cys Asn Leu Gly Val
 70 75 80 85
 GCC ATC GTC TCC ATG GTC AAT AAC AGC ACG ACC CAC CGC GGG GGC CAC 763
 Ala Ile Val Ser Met Val Asn Asn Ser Thr Thr His Arg Gly Gly His
 90 95 100
 GTG GTG GTG CAG AAA GCC CAG TTC AGC TGG GAT CCA GAG ACT GTC GGC 811
 Val Val Val Gln Lys Ala Gln Phe Ser Trp Asp Pro Glu Thr Val Gly
 105 110 115
 CTC ATA CAC GGC TCC TTT TTC TGG GGC TAC ATT GTC ACT CAG ATT CCA 859
 Leu Ile His Gly Ser Phe Phe Trp Gly Tyr Ile Val Thr Gln Ile Pro
 120 125 130

GGA GGA TTT ATC TGT CAA AAA TTT GCA GCC AAC AGA GTT TTC GGC TTT Gly Gly Phe Ile Cys Gln Lys Phe Ala Ala Asn Arg Val Phe Gly Phe 135 140 145	907
GCT ATT GTG GCA ACA TCC ACT CTA AAC ATG CTG ATC CCC TCA GCT GCC Ala Ile Val Ala Thr Ser Thr Leu Asn Met Leu Ile Pro Ser Ala Ala 150 155 160 165	955
CGC GTC CAC TAT GGC TGT GTC ATC TTC GTG AGG ATC CTG CAG GGG TTG Arg Val His Tyr Gly Cys Val Ile Phe Val Arg Ile Leu Gln Gly Leu 170 175 180	1003
GTA GAG GGG GTC ACA TAC CCC GCC TGC CAT GGG ATC TGG AGC AAA TGG Val Glu Gly Val Thr Tyr Pro Ala Cys His Gly Ile Trp Ser Lys Trp 185 190 195	1051
GCC CCA CCC TTA GAA CGG AGT CGC CTG GCG ACG ACA GCC TTT TGT GGT Ala Pro Pro Leu Glu Arg Ser Arg Leu Ala Thr Thr Ala Phe Cys Gly 200 205 210	1099
TCC TAT GCT GGG GCG GTG GTC GCG ATG CCC CTC GCC GGG GTC CTT GTG Ser Tyr Ala Gly Ala Val Ala Met Pro Leu Ala Gly Val Leu Val 215 220 225	1147
CAG TAC TCA GGA TGG AGC TCT GTT TTC TAC GTC TAC GGC AGC TTC GGG Gln Tyr Ser Gly Trp Ser Ser Val Phe Tyr Val Tyr Gly Ser Phe Gly 230 235 240 245	1195
ATC TTC TGG TAC CTG TTC TGG CTG CTC GTC TCC TAC GAG TCC CCC GCG Ile Phe Trp Tyr Leu Phe Trp Leu Leu Val Ser Tyr Glu Ser Pro Ala 250 255 260	1243
CTG CAC CCC AGC ATC TCG GAG GAG CGC AAG TAC ATC GAG GAC GCC Leu His Pro Ser Ile Ser Glu Glu Glu Arg Lys Tyr Ile Glu Asp Ala 265 270 275	1291
ATC GGA GAG AGC GCG AAA CTC ATG AAC CCC CTC ACG AAG TTT AGC ACT Ile Gly Glu Ser Ala Lys Leu Met Asn Pro Leu Thr Lys Phe Ser Thr 280 285 290	1339
CCC TGG CGG CGC TTC TTC ACG TCT ATG CCA GTC TAT GCC ATC ATC GTG Pro Trp Arg Arg Phe Phe Thr Ser Met Pro Val Tyr Ala Ile Ile Val 295 300 305	1387
GCC AAC TTC TGC CGC AGC TGG ACG TTC TAC CTG CTG CTC ATC TCC CAG Ala Asn Phe Cys Arg Ser Trp Thr Phe Tyr Leu Leu Leu Ile Ser Gln 310 315 320 325	1435
CCC GAC TAC TTC GAA GAA GTG TTC GGC TTC GAG ATC AGC AAG GTA GGC Pro Asp Tyr Phe Glu Glu Val Phe Gly Phe Glu Ile Ser Lys Val Gly 330 335 340	1483
CTG GTG TCC GCG CTG CCC CAC CTG GTC ATG ACC ATC ATC GTG CCC ATC Leu Val Ser Ala Leu Pro His Leu Val Met Thr Ile Ile Val Pro Ile 345 350 355	1531

GGC GGC CAG ATC GCG GAC TTC CTG CGG AGC CGC CGC ATC ATG TCC ACC Gly Gly Gln Ile Ala Asp Phe Leu Arg Ser Arg Arg Ile Met Ser Thr 360 365 370	1579
ACC AAC GTG CGC AAG TTG ATG AAC TGC GGA GGC TTC GGC ATG GAA GCC Thr Asn Val Arg Lys Leu Met Asn Cys Gly Gly Phe Gly Met Glu Ala 375 380 385	1627
ACG CTG CTG TTG GTG GTC GGC TAC TCG CAC TCC AAG GGC GTG GCC ATC Thr Leu Leu Leu Val Val Gly Tyr Ser His Ser Lys Gly Val Ala Ile 390 395 400 405	1675
TCC TTC CTG GTC CTA GCC GTG GGC TTC AGC GGC TTC GCC ATC TCT GGG Ser Phe Leu Val Leu Ala Val Gly Phe Ser Gly Phe Ala Ile Ser Gly 410 415 420	1723
TTC AAC GTG AAC CAC CTG GAC ATA GCC CCG CGC TAC GCC AGC ATC CTC Phe Asn Val Asn His Leu Asp Ile Ala Pro Arg Tyr Ala Ser Ile Leu 425 430 435	1771
ATG GGC ATC TCC AAC GGC GTG GGC ACA CTG TCG GGC ATG GTG TGC CCC Met Gly Ile Ser Asn Gly Val Gly Thr Leu Ser Gly Met Val Cys Pro 440 445 450	1819
ATC ATC GTG GGG GCC ATG ACT AAG CAC AAG ACT CGG GAG GAG TGG CAG Ile Ile Val Gly Ala Met Thr Lys His Lys Thr Arg Glu Glu Trp Gln 455 460 465	1867
TAC GTG TTC CTA ATT GCC TCC CTG GTG CAC TAT GGA GGT GTC ATC TTC Tyr Val Phe Leu Ile Ala Ser Leu Val His Tyr Gly Gly Val Ile Phe 470 475 480 485	1915
TAC GGG GTC TTT GCT TCT GGA GAG AAG CAG CCG TGG GCA GAG CCT GAG Tyr Gly Val Phe Ala Ser Gly Glu Lys Gln Pro Trp Ala Glu Pro Glu 490 495 500	1963
GAG ATG AGC GAG GAG AAG TGT GGC TTC GTT GGC CAT GAC CAG CTG GCT Glu Met Ser Glu Glu Lys Cys Gly Phe Val Gly His Asp Gln Leu Ala 505 510 515	2011
GGC AGT GAC GAC AGC GAA ATG GAG GAT GAG GCT GAG CCC CCG GGG GCA Gly Ser Asp Asp Ser Glu Met Glu Asp Glu Ala Glu Pro Pro Gly Ala 520 525 530	2059
CCC CCT GCA CCC CCG CCC TCC TAT GGG GCC ACA CAC AGC ACA TTT CAG Pro Pro Ala Pro Pro Ser Tyr Gly Ala Thr His Ser Thr Phe Gln 535 540 545	2107
CCC CCC AGG CCC CCA CCC CCT GTC CGG GAC TAC TGA CCATGTGCCT Pro Pro Arg Pro Pro Pro Val Arg Asp Tyr * 550 555 560	2153
CCCACTGAAT GGCAGTTTCC AGGACCTCCA TTCCACTCAT CTCTGGCCTG AGTGACAGTG TCAAGGAACC CTGCTCCTCT CTGTCCTGCC TCAGGCCTAA GAAGCACTCT CCCTTGTTC	2213
	2273

CAGTGCTGTC AAATCCTCTT TCCTTCCCAA TTGCCTCTCA GGGTAGTGA AGCTGCAGAC	2333
TGACAGTTTC AAGGATAACCC AAATTCCCTT AAAGGTTCCC TCTCCACCCG TTCTGCCTCA	2393
GTGGTTTCAA ATCTCTCCTT TCAGGGCTTT ATTTGAATGG ACAGTTCGAC CTCTTACTCT	2453
CTCTTGTGGT TTTGAGGCAC CCACACCCCC CGCTTCCCTT TATCTCCAGG GACTCTCAGG	2513
CTAACCTTTG AGATCACTCA GCTCCCACCT CCTTTCAGAA AAATTCAAGG TCCTCCTCTA	2573
GAAGTTTCAA ATCTCTCCCA ACTCTGTTCT GCATCTTCICA GATGGTTTA ACCAATTACT	2633
CGTCCCCGCC ATTCCAGGGA TTGATTCTCA CCAGCGTTTC TGATGGAAAA TGGCGGAAAT	2693
TCCTGCAGCC CGGGGGATCC ACT	2716

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 561 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Phe Arg Gln Glu Glu Phe Arg Lys Leu Ala Gly Arg Ala Leu	
1 5 10 15	
Gly Lys Leu His Arg Leu Leu Glu Lys Arg Gln Glu Gly Ala Glu Thr	
20 25 30	
Leu Glu Leu Ser Ala Asp Gly Arg Pro Val Thr Thr Gln Thr Arg Asp	
35 40 45	
Pro Pro Val Val Asp Cys Thr Cys Phe Gly Leu Pro Arg Arg Tyr Ile	
50 55 60	
Ile Ala Ile Met Ser Gly Leu Gly Phe Cys Ile Ser Phe Gly Ile Arg	
65 70 75 80	
Cys Asn Leu Gly Val Ala Ile Val Ser Met Val Asn Asn Ser Thr Thr	
85 90 95	
His Arg Gly Gly His Val Val Val Gln Lys Ala Gln Phe Ser Trp Asp	
100 105 110	
Pro Glu Thr Val Gly Leu Ile His Gly Ser Phe Phe Trp Gly Tyr Ile	
115 120 125	
Val Thr Gln Ile Pro Gly Gly Phe Ile Cys Gln Lys Phe Ala Ala Asn	
130 135 140	

Arg Val Phe Gly Phe Ala Ile Val Ala Thr Ser Thr Leu Asn Met Leu
145 150 155 160

Ile Pro Ser Ala Ala Arg Val His Tyr Gly Cys Val Ile Phe Val Arg
165 170 175

Ile Leu Gln Gly Leu Val Glu Gly Val Thr Tyr Pro Ala Cys His Gly
180 185 190

Ile Trp Ser Lys Trp Ala Pro Pro Leu Glu Arg Ser Arg Leu Ala Thr
195 200 205

Thr Ala Phe Cys Gly Ser Tyr Ala Gly Ala Val Val Ala Met Pro Leu
210 215 220

Ala Gly Val Leu Val Gln Tyr Ser Gly Trp Ser Ser Val Phe Tyr Val
225 230 235 240

Tyr Gly Ser Phe Gly Ile Phe Trp Tyr Leu Phe Trp Leu Leu Val Ser
245 250 255

Tyr Glu Ser Pro Ala Leu His Pro Ser Ile Ser Glu Glu Glu Arg Lys
260 265 270

Tyr Ile Glu Asp Ala Ile Gly Glu Ser Ala Lys Leu Met Asn Pro Leu
275 280 285

Thr Lys Phe Ser Thr Pro Trp Arg Arg Phe Phe Thr Ser Met Pro Val
290 295 300

Tyr Ala Ile Ile Val Ala Asn Phe Cys Arg Ser Trp Thr Phe Tyr Leu
305 310 315 320

Leu Leu Ile Ser Gln Pro Asp Tyr Phe Glu Glu Val Phe Gly Phe Glu
325 330 335

Ile Ser Lys Val Gly Leu Val Ser Ala Leu Pro His Leu Val Met Thr
340 345 350

Ile Ile Val Pro Ile Gly Gly Gln Ile Ala Asp Phe Leu Arg Ser Arg
355 360 365

Arg Ile Met Ser Thr Thr Asn Val Arg Lys Leu Met Asn Cys Gly Gly
370 375 380

Phe Gly Met Glu Ala Thr Leu Leu Leu Val Val Gly Tyr Ser His Ser
385 390 395 400

Lys Gly Val Ala Ile Ser Phe Leu Val Leu Ala Val Gly Phe Ser Gly
405 410 415

Phe Ala Ile Ser Gly Phe Asn Val Asn His Leu Asp Ile Ala Pro Arg
420 425 430

Tyr Ala Ser Ile Leu Met Gly Ile Ser Asn Gly Val Gly Thr Leu Ser
 435 440 445

Gly Met Val Cys Pro Ile Ile Val Gly Ala Met Thr Lys His Lys Thr
 450 455 460

Arg Glu Glu Trp Gln Tyr Val Phe Leu Ile Ala Ser Leu Val His Tyr
 465 470 475 480

Gly Gly Val Ile Phe Tyr Gly Val Phe Ala Ser Gly Glu Lys Gln Pro
 485 490 495

Trp Ala Glu Pro Glu Glu Met Ser Glu Glu Lys Cys Gly Phe Val Gly
 500 505 510

His Asp Gln Leu Ala Gly Ser Asp Asp Ser Glu Met Glu Asp Glu Ala
 515 520 525

Glu Pro Pro Gly Ala Pro Pro Ala Pro Pro Pro Ser Tyr Gly Ala Thr
 530 535 540

His Ser Thr Phe Gln Pro Pro Arg Pro Pro Pro Pro Val Arg Asp Tyr
 545 550 555 560

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2716 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGAUAAAGCUU GAUAUCGAAU UCCGGACUCU UGCUCGGCG CCUUAACCG GCGUUCGGUU	60
CAUCCCGCAG CGCCAGUUCU GCUUACCAAA AGUGGCCAC UAGGCACUCG CAUUCCACGC	120
CCGGCUCCAC GCCAGCGAGC CGGGCUUCUU ACCCAUUUAA AGUUUGAGAA UAGGUUGAGA	180
UCGUUUCGGC CCCAAGACCU CUAAUCAUUC GCUUUACCGG AUAAAACUGC GUUGCGGGGG	240
UGCGUCGGGU CUGCGAGAGC GCCAGCUAUC CUGAGGGAAA CUUCGGAGGG AACCAGCUAC	300
UAGAUGGUUC GAUUAGUCUU UCGCCCCUAU ACCCAGGUCG GACGACCGAU UUGCACGUCA	360
GGACCGCUAC GGACCUCCAC CAGAGUUUCC UCUGGCUUCG CCCUGCCCAG GCGAUCCGG	420

GGGGGGACCC	GCAGGGUGAC	CGGCGGCAGG	AGCCGCCACC	AUGGAGUUCC	GCCAGGAGGA	480
GUUUUCGGAAG	CUAGCGGGUC	GUGCUCUCGG	GAAGCUGCAC	CGCCUUCUGG	AGAAGCGGCA	540
GGAAGGCGCG	GAGACGCUGG	AGCUGAGUGC	GGAUGGGCGC	CCGGUGACCA	CGCAGACCCG	600
GGACCCGCG	GUGGUGGACU	GCACCUGCUU	CGGCCUCCCCU	CGCCGCUACA	UUAUCCAU	660
CAUGAGUGGU	CUGGGCUUCU	GCAUCAGCUU	UGGCAUCCGC	UGCAACCUGG	GCGUGGCCAU	720
CGUCUCCAUG	GUAAUAACA	GCACGACCCA	CCGCGGGGGC	CACGUGGUGG	UGCAGAAAGC	780
CCAGUUCAGC	UGGGAUCCAG	AGACUGUCGG	CCUCAUACAC	GGCUCCUUUU	UCUGGGCUA	840
CAUUGUCACU	CAGAUUCCAG	GAGGAUUUAU	CUGUAAAAAA	UUUGCAGCCA	ACAGAGUUUU	900
CGGCUUUGCU	AUUGUGGCAA	CAUCCACUCU	AAACAUGCUG	AUCCCCUCAG	CUGCCCGCGU	960
CCACUAUGGC	UGUGUCAUCU	UCGUGAGGAU	CCUGCAGGGG	UUGGUAGAGG	GGGUACACAU	1020
CCCCGCCUGC	CAUGGGAUCU	GGAGCAAAUG	GGCCCCACCC	UUAGAACGGA	GUCGCCUGGC	1080
GACGACAGCC	UUUUGUGGUU	CCUAUGCUGG	GGCGGUGGUC	GCGAUGCCCC	UCGCCGGGU	1140
CCUUGUGCAG	UACUCAGGAU	GGAGCUCUGU	UUUCUACGUC	UACGGCAGCU	UCGGGAUCUU	1200
CUGGUACCUG	UUCUGGGCUGC	UCGUCUCCUA	CGAGUCCCCC	GCGCUGCACC	CCAGCAUCUC	1260
GGAGGGAGGAG	CGCAAGUACA	UCGAGGACGC	CAUCGGAGAG	ACCGCGAAC	UCAUGAACCC	1320
CCUCACGAAG	UUUAGCACUC	CCUGGGCGCG	CUUCUUCACG	UCUAUGCCAG	UCUAUGCCAU	1380
CAUCGUGGCC	AACUUCUGCC	GCAGCUGGAC	GUUCUACCUG	CUGCUCAUCU	CCCAGCCCCGA	1440
CUACUUCGAA	GAAGUGUUCG	GCUUCGAGAU	CAGCAAGGUA	GGCCUGGUGU	CCGCGCUGCC	1500
CCACCUGGUC	AUGACCAUCA	UCGUGCCCAU	CGGCGGCCAG	AUCGCGGACU	UCCUGCGGAG	1560
CCGCCGCAUC	AUGUCCACCA	CCAACGUGCG	CAAGUJGAUG	AACUGCGGAG	GCUUCCGCAU	1620
GGAAGCCACG	CUGCUGUJUGG	UGGUCCGCUA	CUCGCACUCC	AAGGGCGUGG	CCAUCUCCUU	1680
CCUGGUCCUA	GGCGUGGGCU	UCAGGGCUU	CGCCAUCUCU	GGGUUCAACG	UGAACCAACCU	1740
GGACAUAGCC	CCGCGCUACG	CCAGCAUCCU	CAUGGGCAUC	UCCAACGGCG	UGGGCACACU	1800
GUCGGGCAUG	GUGUGCCCCA	UCAUCGUGGG	GGCCAUGACU	AAGCACAAGA	CUCGGGAGGA	1860
GUGGCAGUAC	GUGUUCCUA	UUGCCUCCCCU	GGUGCACUUAU	GGAGGUGUCA	UCUUCUACGG	1920
GGUCUUUGCU	UCUGGAGAGA	AGCAGCCGUG	GGCAGAGCCU	GAGGAGAUGA	GCGAGGAGAA	1980
GUGUGGUUC	GUUGGCCAUG	ACCAGCUGGC	UGGCAGUGAC	GACAGCGAAA	UGGAGGAUGA	2040

GGCUGAGCCC	CCGGGGCAC	CCCCUGCACC	CCCGCCCUCC	UAUGGGCCA	CACACAGCAC	2100
AUUCAGCCC	CCCAGGCC	CACCCCUGU	CCGGGACUAC	UGACCAUGUG	CCUCCCACUG	2160
AAUGGCAGUU	UCCAGGACCU	CCAUUCCACU	CAUCUCUGGC	CUGAGUGACA	GUGUCAAGGA	2220
ACCCUGCUCC	UCUCUGUCCU	GCCUCAGGCC	UAAGAACAC	UCUCCUUGU	UCCCAGUGCU	2280
GUCAAAUCCU	CUUCCUUCC	CAAUUGCCUC	UCAGGGGUAG	UGAAGCUGCA	GACUGACAGU	2340
UUCAAGGAUA	CCCAAAUUCC	CCUAAAGGUU	CCCUCUCCAC	CCGUUCUGCC	UCAGUGGUUU	2400
CAAAUCUC	CUUUCAGGGC	UUUAUUUGAA	UGGACAGUUC	GACCUCUUAC	UCUCUCUUGU	2460
GGUUUUGAGG	CACCCACACC	CCCCGCUUUC	CUUUAUCUCC	AGGGACUCUC	AGGCUAACCU	2520
UUGAGAUCAC	UCAGCUCCCA	UCUCCUUUCA	GAAAAAUUCA	AGGUCCUCCU	CUAGAAGUUU	2580
CAAAUCUC	CCAACUCUGU	UCUGCAUCUU	CCAGAUUGGU	UUAACCAAUU	ACUCGUCCCC	2640
GCCAUUCCAG	GGAUUGAUUC	UCACCAGCGU	UUCUGAUGGA	AAAUGGCCGG	AAUUCCUGCA	2700
GCCCCGGGGGA	UCCACU					2716

We Claim:

1. An isolated amino acid compound functional as
a human brain Na⁺-dependent inorganic phosphate cotransporter
5 which comprises the amino acid sequence

Met Glu Phe Arg Gln Glu Glu Phe Arg Lys Leu Ala Gly Arg Ala Leu
1 5 10 15

10 Gly Lys Leu His Arg Leu Leu Glu Lys Arg Gln Glu Gly Ala Glu Thr
20 25 30

Leu Glu Leu Ser Ala Asp Gly Arg Pro Val Thr Thr Gln Thr Arg Asp
15 35 40 45

Pro Pro Val Val Asp Cys Thr Cys Phe Gly Leu Pro Arg Arg Tyr Ile
50 55 60

Ile Ala Ile Met Ser Gly Leu Gly Phe Cys Ile Ser Phe Gly Ile Arg
20 65 70 75 80

Cys Asn Leu Gly Val Ala Ile Val Ser Met Val Asn Asn Ser Thr Thr
85 90 95

His Arg Gly Gly His Val Val Val Gln Lys Ala Gln Phe Ser Trp Asp
25 100 105 110

Pro Glu Thr Val Gly Leu Ile His Gly Ser Phe Phe Trp Gly Tyr Ile
30 115 120 125

Val Thr Gln Ile Pro Gly Gly Phe Ile Cys Gln Lys Phe Ala Ala Asn
130 135 140

Arg Val Phe Gly Phe Ala Ile Val Ala Thr Ser Thr Leu Asn Met Leu
35 145 150 155 160

Ile Pro Ser Ala Ala Arg Val His Tyr Gly Cys Val Ile Phe Val Arg
165 170 175

Ile Leu Gln Gly Leu Val Glu Gly Val Thr Tyr Pro Ala Cys His Gly
40 180 185 190

Ile Trp Ser Lys Trp Ala Pro Pro Leu Glu Arg Ser Arg Leu Ala Thr
45 195 200 205

Thr Ala Phe Cys Gly Ser Tyr Ala Gly Ala Val Val Ala Met Pro Leu
210 215 220

Ala Gly Val Leu Val Gln Tyr Ser Gly Trp Ser Ser Val Phe Tyr Val
50 225 230 235 240

Tyr Gly Ser Phe Gly Ile Phe Trp Tyr Leu Phe Trp Leu Leu Val Ser
 245 250 255

5 Tyr Glu Ser Pro Ala Leu His Pro Ser Ile Ser Glu Glu Glu Arg Lys
 260 265 270

Tyr Ile Glu Asp Ala Ile Gly Glu Ser Ala Lys Leu Met Asn Pro Leu
 275 280 285

10 Thr Lys Phe Ser Thr Pro Trp Arg Arg Phe Phe Thr Ser Met Pro Val
 290 295 300

Tyr Ala Ile Ile Val Ala Asn Phe Cys Arg Ser Trp Thr Phe Tyr Leu
 305 310 315 320

15 Leu Leu Ile Ser Gln Pro Asp Tyr Phe Glu Glu Val Phe Gly Phe Glu
 325 330 335

20 Ile Ser Lys Val Gly Leu Val Ser Ala Leu Pro His Leu Val Met Thr
 340 345 350

Ile Ile Val Pro Ile Gly Gly Gln Ile Ala Asp Phe Leu Arg Ser Arg
 355 360 365

25 Arg Ile Met Ser Thr Thr Asn Val Arg Lys Leu Met Asn Cys Gly Gly
 370 375 380

Phe Gly Met Glu Ala Thr Leu Leu Leu Val Val Gly Tyr Ser His Ser
 385 390 395 400

30 Lys Gly Val Ala Ile Ser Phe Leu Val Leu Ala Val Gly Phe Ser Gly
 405 410 415

35 Phe Ala Ile Ser Gly Phe Asn Val Asn His Leu Asp Ile Ala Pro Arg
 420 425 430

Tyr Ala Ser Ile Leu Met Gly Ile Ser Asn Gly Val Gly Thr Leu Ser
 435 440 445

40 Gly Met Val Cys Pro Ile Ile Val Gly Ala Met Thr Lys His Lys Thr
 450 455 460

Arg Glu Glu Trp Gln Tyr Val Phe Leu Ile Ala Ser Leu Val His Tyr
 465 470 475 480

45 Gly Gly Val Ile Phe Tyr Gly Val Phe Ala Ser Gly Glu Lys Gln Pro
 485 490 495

50 Trp Ala Glu Pro Glu Glu Met Ser Glu Glu Lys Cys Gly Phe Val Gly
 500 505 510

His Asp Gln Leu Ala Gly Ser Asp Asp Ser Glu Met Glu Asp Glu Ala
 515 520 525

55 Glu Pro Pro Gly Ala Pro Pro Ala Pro Pro Ser Tyr Gly Ala Thr

530 535 540

His Ser Thr Phe Gln Pro Pro Arg Pro Pro Pro Val Arg Asp Tyr
545 550 555 560

5

which is SEQ ID NO:2, or a functional equivalent thereof, or a fragment of at least 6 continuous amino acids thereof.

10 2. A nucleic acid compound encoding an amino acid compound of Claim 1.

15 3. A composition comprising an isolated nucleic acid compound containing a sequence encoding a human brain Na⁺-dependent inorganic phosphate cotransporter or fragment thereof as claimed in Claim 2, wherein said sequence encoding a human brain Na⁺-dependent inorganic phosphate cotransporter or fragment thereof is selected from the group consisting of:

20 (a) CGATAAGCTT GATATCGAAT TCCGGACTCT TGCTCGGGCG CCTTAACCCG GCGTCGGTT
CATCCCGCAG CGCCAGTTCT GCTTACCAAA AGTGGCCAC TAGGCACTCG CATTCCACGC
CCGGCTCCAC GCCAGCGAGC CGGGCTTCTT ACCCATTAA AGTTTGAGAA TAGTTGAGA
25 TCGTTTCGGC CCCAAGACCT CTAATCATTG GCTTTACCGG ATAAAATGCG GTGGCGGGGG
TGCGTCGGGT CTGCGAGAGC GCCAGCTATC CTGAGGGAAA CTTCGGAGGG AACCAAGCTAC
30 TAGATGGTTC GATTAGTCTT TCGCCCCTAT ACCCAGGTGCG GACGACCGAT TTGACAGTCA
GGACCGCTAC GGACCTCCAC CAGAGTTCC TCTGGCTTCG CCCTGCCAG GCGATCGGCG
35 GGGGGGACCC GCGGGGTGAC CGGCGGCAGG AGCCGCCACC ATGGAGTTCC GCCAGGAGGA
GTTTCGGAAG CTAGCGGGTC GTGCTCTCGG GAAGCTGCAC CGCCTCTGG AGAACGGGCA
GGAAGGCGCG GAGACGCTGG AGCTGAGTGC GGATGGCGC CCGGTGACCA CGCAGACCCG
40 GGACCCGCCG GTGGTGGACT GCACCTGCTT CGGCCTCCCT CGCCGCTACA TTATGCCAT
CATGAGTGGT CTGGGCTTCT GCATCAGCTT TGGCATCCGC TGCAACCTGG GCGTGGCCAT
CGTCTCCATG GTCAATAACA GCACGACCCA CCGCGGGGGC CACGTGGTGG TGCAGAAAGC
45 CCAGTTCAGC TGGGATCCAG AGACTGTCGG CCTCATACAC GGCTCCTTTT TCTGGGGCTA
CATTGTCACT CAGATTCCAG GAGGATTAT CTGTAAAAAA TTTGCAGCCA ACAGAGTTT

CGGCTTGCT ATTGTGGCAA CATCCACTCT AAACATGCTG ATCCCCTCAG CTGCCCGCGT
5 CCACTATGGC TGTGTCATCT TCGTGAGGAT CCTGCAGGGG TTGGTAGAGG GGTCACATA
CCCCGCCTGC CATGGATCT GGAGCAAATG GGCCCCACCC TTAGAACGGA GTGCCCTGGC
GACGACAGCC TTTTGTGGTT CCTATGCTGG GCGGGTGGTC GCGATGCCCC TCGCCGGGGT
10 CCTTGTGCAG TACTCAGGAT GGAGCTCTGT TTTCTACGTC TACGGCAGCT TCGGGATCTT
CTGGTACCTG TTCTGGCTGC TCGTCTCCTA CGAGTCCCCC GCGCTGCACC CCAGCATCTC
GGAGGAGGAG CGCAAGTACA TCGAGGACGC CATCGGAGAG AGCGCGAAAC TCATGAACCC
15 CCTCACGAAG TTTAGCACTC CCTGGCGGG CTTCTTCACG TCTATGCCAG TCTATGCCAT
CATCGTGGCC AACTTCTGCC GCAGCTGGAC GTTCTACCTG CTGCTCATCT CCCAGCCGA
20 CTACTTCGAA GAAGTGTTCG GCTTCGAGAT CAGCAAGGTA GCCCTGGTGT CGCGCTGCC
CCACCTGGTC ATGACCATCA TCGTGCCCAT CGGCGGCCAG ATCGCGGACT TCCTGCGGAG
25 CCGCCGCATC ATGTCCACCA CCAACGTGCG CAAGTTGATG AACTGCGGAG GCTTCCGCAT
GGAAGCCACG CTGCTGTTGG TGTCGGCTA CTCGCACTCC AAGGGCGTGG CCATCTCCTT
CCTGGTCCTA GCCGTGGGCT TCAGCGGCTT CGCCATCTCT GGGTTCAACG TGAACCACCT
30 GGACATAGCC CCGCGCTACG CCAGCATCCT CATGGCATC TCCAACGGCG TGGGCACACT
GTGGGGCATG GTGTCCCCA TCATCGTGGG GGCCATGACT AAGCACAAGA CTCGGGAGGA
GTGGCAGTAC GTGTTCTAA TTGCTCCCT GGTGCACTAT GGAGGTGTCA TCTTCTACGG
35 GGTCTTGCT TCTGGAGAGA AGCAGCCGTG GGCAGAGCCT GAGGAGATGA GCGAGGAGAA
GTGTGGCTTC GTTGGCCATG ACCAGCTGGC TGGCAGTGAC GACAGCGAAA TGGAGGATGA
40 GGCTGAGCCC CCGGGGGCAC CCCCTGCACC CCCGCCCTCC TATGGGGCCA CACACAGCAC
ATTTCAGCCC CCCAGGGCCC CACCCCTGT CCGGGACTAC TGACCATGTG CCTCCCACTG
45 AATGGCAGTT TCCAGGACCT CCATTCCACT CATCTCTGGC CTGAGTGACA GTGTCAAGGA
ACCCTGCTCC TCTCTGTCCT GCCTCAGGCC TAAGAACAC TCTCCCTGT TCCCAGTGCT
GTCAAATCCT CTTCCTTCC CAATTGCCTC TCAGGGTAG TGAAGCTGCA GACTGACAGT
50 TTCAAGGATA CCCAAATTCC CCTAAAGGTT CCCTCTCCAC CGTTCTGCC TCAGTGGTTT
CAAATCTCTC CTTTCAGGGC TTTATTTGAA TGGACAGTTC GACCTCTTAC TCTCTCTTGT
GGTTTGAGG CACCCACACC CCCCCTTTC CTTTATCTCC AGGGACTCTC AGGCTAACCT
55

TTGAGATCAC TCAGCTCCC A TCTCCTTC GAAAAATTCA AGGTCTCC CTAGAAGTT
CAAATCTCTC CCAACTCTGT TCTGCATCTT CCAGATTGGT TTAACCAATT ACTCGTCCCC
5 GCCATTCCAG GGATTGATTC TCACCAGCGT TTCTGATGGA AAATGGCGGG AATTCTGCA
GCCCGGGGGA TCCACT

which is SEQ ID NO:1;

10 (b) CGAUAAAGCUU GAUAUCGAAU UCCGGACUCU UGCUCGGCG CCUUAAACCCG GCGUUCGGUU
CAUCCCGCAG CGCCAGUUCU GCUUACCAAA AGUGGCCAC UAGGCACUCG CAUCCACGC
15 CCGGCUCCAC GCCAGCGAGC CGGGCUUCUU ACCCAUUUA AGUUUGAGAA UAGGUUGAGA
UCGUUUCGGC CCCAAGACCU CUAUCAUUC GCUUUACCGG AUAAAACUGC GUGGCGGGGG
UGCGUCGGGU CUGCGAGAGC GCCAGCUAUC CUGAGGGAAA CUUCGGAGGG AACCAAGCUAC
20 UAGAUGGUUC GAUUAGUCUU UCGCCCCUAU ACCCAGGUUC GACGACCGAU UUGCACGUCA
GGACCGCUAC GGACCUCCAC CAGAGUUUCC UCUGGUUCG CCCUGCCAG GCGAUCGGCG
25 GGGGGACCC GCGGGUGAC CGGCGGCAGG AGCCGCCACC AUGGAGUUCC GCCAGGAGGA
GUUUCGGAAG CUAGCGGGUC GUGCUCUCGG GAAGCUGCAC CGCCUUCUGG AGAAGCGGCA
30 GGAAGGCGCG GAGACGCUGG AGCUGAGUGC GGAUGGGCGC CCGGUGACCA CGCAGACCCG
GGACCCGCCG GUGGUGGACU GCACCUGCUU CGGCCUCCCU CGCCGUACA UUAUCGCCAU
CAUGAGUGGU CUGGGCUUCU GCAUCAGCUU UGGCAUCCGC UGCAACCUGG GCGUGGCCAU
35 CGUCUCCAUG GUAAUAACA GCACGACCCCA CCGCGGGGGC CACGUGGUGG UGCAGAAAGC
CCAGUUCAGC UGGGAUCCAG AGACUGUCGG CCUCAUACAC GGCUCUUUU UCUGGGCUC
40 CAUJUGUCACU CAGAUUCCAG GAGGAUUUAU CUGUAAAAAA UUUGCAGCCA ACAGAGUUUU
CGGCUUUGCUC AUUGUGGCCAA CAUCCACUCU AAACAUGCUG AUCCCCUCAG CUGCCCGCGU
CCACUAUGGC UGUGUCAUCU UCGUGAGGAU CCUGCAGGGG UUGGUAGAGG GGGUCACAU
45 CCCCCCUGC CAUGGGAUUC GGAGCAAAUG GGCCCCACCC UUAGAACGGA GUCGCCUGGC
GACGACAGCC UUUUGUGGUU CCUAUGCUUGG GGCUGGUUGUC GCGAUGCCCC UCGCCGGGU
50 CCUUGUGCAG UACUCAGGAU GGAGCUCUGU UUUCUACGUC UACGGCAGCU UCGGGAUUCU
CUGGUACCUG UUCUGGCUGC UCGUCUCCUA CGAGUCCCCC GCGCUGCACC CCAGCAUCUC
GGAGGAGGAG CGCAAGUACA UCGAGGACGC CAUCGGAGAG AGCGCGAAC UCAUGAACCC

CCUCACGAAG UUUAGCACUC CCUGGCGGCG CUUCUUCACG UCUAUGCCAG UCUAUGCCAU
CAUCGUGGCC AACUUCUGCC GCAGCUGGAC GUUCUACCUG CUGCUCAUCU CCCAGCCCAG
5 CUACUUCGAA GAAGUGUUCG GCUUCGAGAU CAGCAAGGUU GGCCUGGUGU CCGCGCUGCC
CCACCUUGGUC AUGACCAUCA UCGUGCCCAU CGGCAGGCCAG AUCGCGGACU UCCUGCGGAG
10 CCGCCGCAUC AUGUCCACCA CCAACGUGCG CAAGUUGAUG AACUGCGGAG GCUUCGGCAU
GGAAGCCACG CUGCUGUUGG UGGUCGGCUA CUCGCACUCC AAGGGCGUGG CCAUCUCCUU
CCUGGUCCUA GCCGUGGGCU UCAGCGGCUU CGCCAUCUCU GGGUUCAACG UGAACCACCU
15 GGACAUAGCC CCGCGCUACG CCAGCAUCCU CAUGGGCAUC UCCAACGGCG UGGGCACACU
GUCCCCAUG GUGUGCCCCA UCAUCGUGGG GGCCAUAGACU AAGCACAAGA CUCGGGAGGA
GUGGCAGUAC GUGUUCCUA UUGCCUCCCU GGUGCACUUAU GGAGGUGUCA UCUUCUACGG
20 GGUUUUGCU UCUGGAGAGA AGCAGCCGUG GGCAGAGCCU GAGGAGAUGA GCGAGGAGAA
GUGUGGCUUC GUUGGCAUG ACCAGCUGGC UGGCAGUGAC GACAGCGAAA UGGAGGAUGA
25 GGCUGAGCCC CCGGGGCAC CCCCUGCACC CCCGCCUCC UAUGGGGCCA CACACAGCAC
AUUUCAGCCC CCCAGGCCCA CACCCCCUGU CGGGACUAC UGACCAUGUG CCUCCACUG
30 AAUGGCAGUU UCCAGGACCU CCAUUCCACU CAUCUCUGGC CUGAGUGACA GUGUCAAGGA
ACCCUGCUCC UCUCUGUCCU GCCUCAGGCC UAAGAACAC UCUCCCUUGU UCCCAGUGCU
GUAAAUCU CUUUCCUCC CAAUUGCCUC UCAGGGGUAG UGAAGCUGCA GACUGACAGU
35 UUCAAGGAUA CCCAAAUUCC CCUAAAGGUU CCCUCUCCAC CCGUUCUGCC UCAGUGGUUU
CAAUUCUCUC CUUUCAGGGC UUUAUUUGAA UGGACAGUUC GACCUCUUAC UCUCUCUUGU
40 GGUUUUGAGG CACCCACACC CCCCACUUUC CUUUAUCUCC AGGGACUCUC AGGCUAACC
UUGAGAUCAC UCAGCUCCCA UCUCUUUCA GAAAAAUUCA AGGUCCUCCU CUAGAAGUUU
CAAUUCUCUC CCAACUCUGU UCUGCAUCUU CCAGAUUGGU UUAACCAAUU ACUCGUCCCC
45 GCCAUUCCAG GGAUUGAUUC UCACCAGCGU UUCUGAUGGA AAAUGGCGGG AAUUCCUGCA
GCCCGGGGGGA UCCACU

which is SEQ ID NO:3;

50

(c) a nucleic acid compound complementary to (a) or
(b); and

5 (d) a fragment of (a), (b), or (c) that is at least 18 bases in length and which will selectively hybridize to human genomic DNA encoding a human brain Na⁺-dependent inorganic phosphate cotransporter.

10 4. An expression vector capable of producing a human brain sodium-dependent inorganic phosphate 10 cotransporter, or a fragment thereof, in a host cell which comprises a nucleic acid compound as claimed in Claim 3 operably linked with regulatory elements necessary for expression of the nucleic acid compound in the host cell.

15 5. An expression vector as claimed in Claim 4 which comprises a nucleic acid compound encompassing nucleotides 461 to 2143 of SEQ ID NO:1, or a sequence complementary to this region.

20 6. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with an inappropriate stimulation of a human Na⁺-dependent inorganic phosphate cotransporter protein which 25 method comprises:

30 a) introducing into a mammalian host cell an expression vector comprising DNA encoding a human hBNPI protein as claimed in either one of Claims 2 or 3;

b) culturing said host cell under conditions such that the human hBNPI protein is expressed;

35 c) exposing said host cell expressing the human hBNPI protein to a test compound; and

5 d) measuring the change in a physiological condition known to be influenced by the binding of native ligand to the human hBNPI protein relative to a control in which the transfected host cell is exposed to native ligand.

10 7. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with an inappropriate stimulation of a human Na⁺-dependent inorganic phosphate cotransporter protein compounds which method comprises:

15 a) introducing into a mammalian host cell an expression vector comprising DNA encoding a human Na⁺-dependent inorganic phosphate cotransporter protein as claimed in either one of Claims 2 or 3;

20 b) culturing said host cell under conditions such that the human Na⁺-dependent inorganic phosphate cotransporter protein is expressed;

25 c) exposing said host cell expressing the human Na⁺-dependent inorganic phosphate cotransporter protein to a test compound;

30 d) exposing said host cell expressing the Na⁺-dependent inorganic phosphate cotransporter protein to inorganic phosphate simultaneously with or following the exposure to the test compound; and

35 e) measuring the change in inorganic phosphate uptake relative to a control in

which the transfected host cell is exposed to only inorganic phosphate.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05792

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 69.1, 252.3, 240.1, 320.1; 530/300, 350; 436/501; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ni et al. Cloning and expression of a cDNA encoding a brain-specific Na-dependent inorganic phosphate cotransporter. Proc. Natl. Acad. Sci. USA. June 1994, Vol. 91, pages 5607-5611, especially pages 5607-5610.	1-5 -----
Y	Chong et al. Molecular Cloning of the cDNA Encoding a Human Renal Sodium Phosphate Transport Protein and Its Assignment to Chromosome 6p21.3-p23. Genomics. November 1993, Vol. 18, pages 355-359, especially pages 355-357.	6, 7
Y	Li et al. Molecular cloning of two rat Na/Pi cotransporters: evidence for differential tissue expression of transcripts. Cellular and Molecular Biology Research. March 1996, Vol. 5, pages 451-460.	1-7
A, P		1-7

Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be part of particular relevance
- "E" earlier document published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- "&" document member of the same patent family

Date of the actual completion of the international search
03 JUNE 1996

Date of mailing of the international search report
15 JUL 1996

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05792

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ni et al. Cloning and expression of a novel cDNA encoding a brain specific Na-dependent inorganic phosphate cotransporter. In: Abstracts of the Society for Neuroscience, 24th Annual Meeting. Volume 20, 1994, Abstract 382.4, page 925.	1-5
Y	Collins et al. Molecular cloning, functional expression, tissue distribution, and in situ hybridization of the renal sodium phosphate (Na/Pi) transporter in the control and hypophosphatemic mouse. FASEB Journal, August 1994, Vol. 8, pages 862-868, especially pages 862-865.	1-7
Y	Magagnin et al. Expression cloning of human and rat renal cortex Na/Pi cotransport. Proc. Natl. Acad. Sci. USA. July 1993, Vol. 90, pages 5979-5983, especially pages 5981-5983.	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05792

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

G01N 33/566; C12P 21/06; C12N 1/20, 15/00; A61K 38/00; C07K 1/00; C07H 21/02

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/7.1, 69.1, 252.3, 240.1, 320.1; 530/300, 350; 436/501; 536/23.1

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN/MEDLINE, EMBASE, BIOSIS, CONFSCI, DISSABS, WPIDS, PATOSEP JICST-EPLUS, APS
search terms: , human brain sodium dependent inorganic phosphate co-transporter, protein, amino acid sequence,
cDNA, recombinant, hBNPI, synonyms and authors

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